EVALUATION OF A RATING SCALE TO INVESTIGATE THE PRESENCE AND ANATOMICAL DISTRIBUTION OF BRAIN MICROBLEEDS IN PATIENTS WITH CEREBROVASCULAR DISEASE

MSc. Clinical Neurology

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I dedicate this work to my loving Abu and Ami whose prayers are always with me.

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Statement of Contributors

Project Design	Dr. David Werring (Supervisor of the Project) Dr. Simone Gregoire (Clinical Research Fellow to David Werring) Dr. Umair Javaid Chaudhary (M.Sc. Clinical Neurology Student)
Project Preparation	Umair Javaid Chaudhary was trained by David Werring (supervisor), Rolf Jager (Consultant Neuroradiologist) and Simone Gregoire to identify brain microbleeds on PACS work stations. The rating scale was designed by Simone Gregoire initially, and was modified in discussion with Umair Javaid Chaudhary and David Werring
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Data Analysis	Dr. Umair Javaid Chaudhary Dr. Simone Gregoire Statistical advice by Dr. Constantinos Kallis
Write up	Dr. Umair Javaid Chaudhary Comments and suggestions from Dr. David Werring and Dr. Simone Gregoire

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Abbreviations

MRI = Magnetic Resonance Imaging

HL = Haemorrhagic Lacunes

ICH = Intracerebral Haemorrhage

CAA = Cerebral Amyloid Angiopathy

GRE = Gradient Recalled Echo

GRE T2*W MRI = Gradient Recalled Echo T2* Weighted Magnetic Resonance Imaging

TIA = Transient Ischemic Attacks

CADASIL = Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts

VD = Vascular Dementia

AD = Alzheimer's disease

CVD = Cerebrovascular Disease

PACS = Picture Archival and Communications System

SVD = Small Vessel Disease

NHNN = National Hospital for Neurology and Neurosurgery

SPSS = Statistical Package for the Social Science

SAH = Subarachnoid Haemorrhage

N = Number

Κ = Kappa Value

TE = Time Echo

TR = Time Repeat

MR = Magnetic Resonance

RF = Radiofrequency

SE = Spin Echo

FSE = Fast Spin Echo

SWI = Susceptibility Weighted Imaging

DWI = Diffusion Weighted Imaging

Abstract

Introduction: *Brain microbleeds are small (do- like) well-defined areas of low signal intensity detectable on GRE T2*W MRI sequences because of the paramagnetic properties of blood breakdown products. Microbleeds are generally considered to range from 2 to 10 mm in size. Histologically they consist of deposits of hemosiderin laden macrophages. Microbleeds are reported both in healthy populations and patients with CVD. They are a risk factor for stroke and may be a marker of SVD including CAA and hypertensive small vessel vasculopathy. Microbleeds are also implicated in the development of cognitive dysfunction and focal neurological clinical symptoms. Perhaps the most important potential clinical question regarding microbleeds is whether they may help in predicting the risk of intracranial bleeding in patients on antithrombotic or thrombolytic treatment. To address all these questions a reliable rating scale is required.* ***Objective:*** *The purpose of this project is to determine intra and inter-rater agreement for the presence, number and anatomical distribution of microbleeds in a cohort of subjects with CVD, using a new rating scale, on two different GRE T2*W MRI sequences.* ***Methods:*** *Out of the initial cohort of 360 unselected, consecutive patients with suspected stroke, 303 patients were included and were divided into two groups based on different TE values for T2*W GRE MRI sequences (TE 40msec, N = 273; TE 26msec, N = 30). Both definite and possible microbleeds were identified, counted and anatomically categorized according to the rating scale by two trained, independent raters. Statistical analysis was performed on SPSS and STATA intercooled.* ***Results:*** *The prevalence of microbleeds was 31 to 38%. Agreement for the presence of definite microbleeds in any location of the brain was good to very good (intra-rater K = 0.87, inter-rater K = 0.69). Agreement for the presence of definite microbleeds in the lobar, deep and infratentorial distribution and individual anatomical regions was also good to very good.* ***Conclusion:*** *This rating scale has good intra and inter-rater agreement for the presence of definite microbleeds in any location of the brain, including the lobar, deep and infratentorial areas and in individual anatomical regions, and should be validated in prospective and cross-sectional multicentre studies.*

Chapter 1: Introduction

1.1: Historical Aspect:

From Charcot-Bouchard Aneurysms to Brain Microbleeds

To understand the nature of microbleeds it is necessary to consider the historical development of concepts regarding small haemorrhagic or aneurysmal lesions near small blood vessels. In 1868 Charcot and Bouchard reported small lesions they termed "miliary parenchymal aneurysms" (Figure 1.1) in patients dying with intracerebral hemorrhage (ICH) (Challa et al. 1992). Subsequently, others confirmed the presence of these lesions and their association with hypertension and ICH (Challa et al. 1992;Cole & Yates 1967;RossRussell 1963). Cole and Yates measured the size of these lesions up to 2mm (Cole & Yates 1967). These lesions have been called Charcot-Bouchard aneurysms, but their exact nature and prevalence remain controversial.

Challa and coworkers used alkaline phosphatase endothelial staining technique and did not find any Charcot Bouchard aneurysms in hypertensive or normotensive cases. Moreover, they rarely found typical parenchymal aneurysms in routine paraffin brain pathological sections from 2800 autopsies. They argued that commonly used previous techniques for studying brain specimens, i.e., intravascular injection of radiopaque contrast media, distends or ruptures the relatively thin walls of brain arteries and leads to artefactual mural outpouchings resembling aneurysms. Secondly, arteriolar twists and coils (Figure 1.2) may also look like aneurysm when injected with contrast media, where-as, the paraffin technique may underestimate the number of aneurysms. They also described that attenuation of muscle cells in the walls of arteries and arterioles, perivascular hemosiderosis and arteriosclerosis, is more prominent in hypertensive subjects than controls (Challa et al. 1992). It is likely

that what we now call "microbleeds" is closely related to the pathological lesions described above, both aneurysmal and non-aneurysmal.

In the modern era, it has become possible to image small vascular lesions in life using certain types of magnetic resonance imaging (MRI) sequences. Scharf and coworkers used magnetic resonance imaging (MRI) to study "haemorrhagic lacunes" (HL), a pathologically proven feature of hypertensive vascular disease. They described the association of HL with ICH. These HL were defined as "lacunar lesions with central or peripheral areas suggesting the presence of haemosiderin-laden macrophages i.e. marked signal loss on T2-weighted images and little signal loss on T1 weighted images" (Scharf et al. 1994). Greenberg and colleagues used gradient recalled echo (GRE) MRI sequences and detected lobar (atleast 1cm in all directions) and petechial hemorrhages (less than 1cm in size) in patients with cerebral amyloid angiopathy (CAA) (Greenberg et al. 1996). In the same year Offenbacher studied 120 patients with a diagnosis of ICH and described round focal areas of homogenous signal loss (2 to 5 mm in size) within brain parenchyma and called them "microbleeds". They used both spin echo (SE) and gradient echo MRI sequences (Offenbacher et al. 1996).

Today, microbleeds are known as areas of tiny collections of blood breakdown products (hemosiderin), which are visualized as small, homogeneous (dot-like), well defined areas of low signal, usually between 2-10mm in diameter on GRE $T2^*$ weighted MRI (GRE T2*W MRI), which are not visible on conventional T2 weighted fast spin echo (T2W FSE) MRI (Roob & Fazekas 2000;Werring 2007), (Figure 1.3).

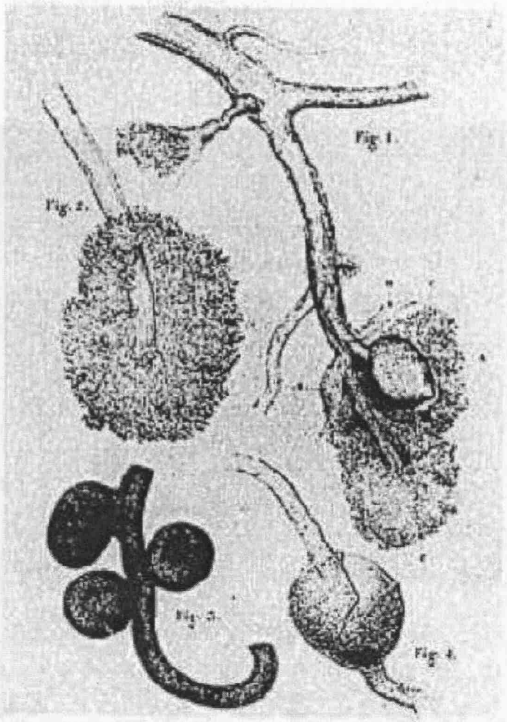


Figure 1.1: Drawings of Charcot Bouchard's aneurysms
"miliary parenchymal aneurysms" by Charcot and Bouchard
(Challa et al. 1992).

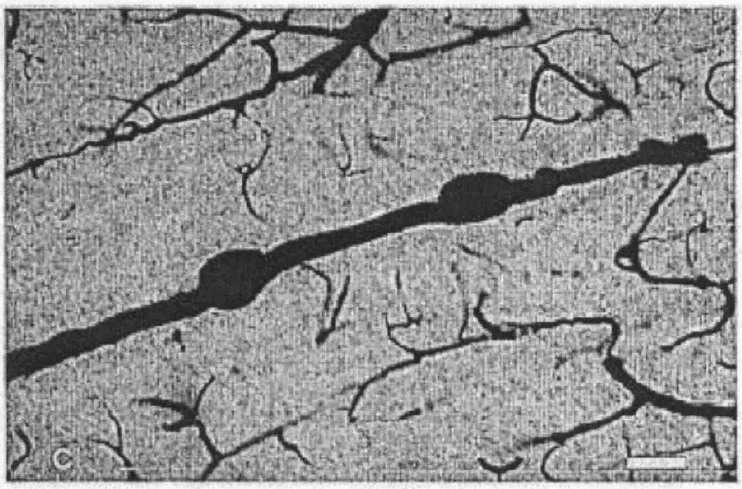


Figure 1.2: Vascular coils and twists
(Challa et al. 1992).

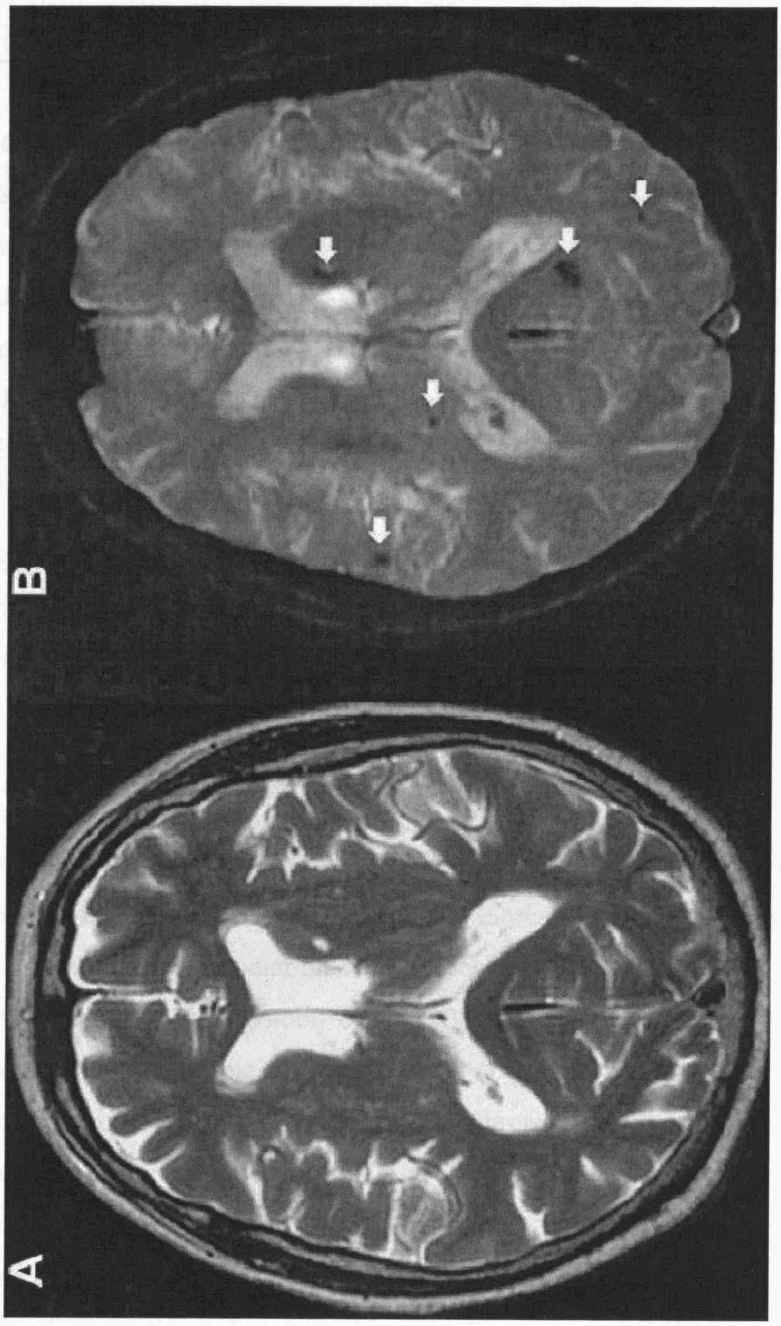


Figure 1.3: Brain Microbleeds on GRE T2*W MRI
Microbleeds (arrow) visible on T2*W GRE-MRI but not visible on T2W SE-MRI
(Werring et al. 2005)

1.2: Burden of Brain Microbleeds

1.2.1: Prevalence

The prevalence of microbleeds has been studied both in community settings and in high risk populations. They were seen in 6.4% of the healthy elderly people in the Austrian stroke prevention study (Roob et al. 1999). The Framingham study (Jeerakathil et al. 2004) and a systematic review (Cordonnier et al. 2007) also confirmed that microbleeds are present in around 5% of the healthy population. More recent studies have shown an even higher prevalence; Sveinbjornsdottir reported a prevalence of microbleeds of 11.1% in a community based sample of healthy people (S S et al. 2008). According to the Rotterdam scan study, microbleed prevalence varies with age from 17.8% (60-69 years) to 38.3% (>80 years). Possible reasons for the higher prevalence in this study, include advanced age of participants of the study and improved quality of GRE MRI used (Vernooij et al. 2008b).

1.2.2: Risk Factors

The prevalence of microbleeds is higher in people with associated cerebrovascular diseases (CVD) and/or certain risk factors. Increasing age has consistently been found to have a strong association with microbleeds (Jeerakathil et al. 2004;Roob et al. 1999;S S et al. 2008;Vernooij et al. 2008b). Two studies found that males had more microbleeds than females (Jeerakathil et al. 2004;S S et al. 2008). Hypertension (elevated systolic blood pressure), lacunar infarcts, extensive white matter lesions (leukoaraiosis), low total serum cholesterol and low-density lipoprotein are other reported independent risk factors (Jeerakathil et al. 2004;Jeong et al. 2004;Lee et al. 2002;Lee et al. 2004a;Roob et al. 1999;Sorimachi et al. 2007;Vernooij et al. 2008b). The risk of microbleeds has been reported to increase 1.8 to 1.9 fold with each standard deviation rise in blood pressure (Henskens et al. 2008). It has also been noted in a case report that despite good control of blood pressure, new microbleeds appear on the follow up MRI scans in a hypertensive patient with ICH (Imaizumi et al. 2003). Thus, although excellent control of hypertension is generally advocated to

decrease the risk of developing further microbleeds, more follow up studies are required to establish the dynamics of microbleeds over time and the effects of blood pressure treatment.

Some risk factors have also shown a differential relationship to the distribution of microbleeds. All areas of the brain may have microbleeds, with a preference for cortico-subcortical areas, followed by the basal ganglia, thalamus and infratentorial regions (Roob et al. 2000;S S et al. 2008). The APOE4 carrier state has been associated with lobar microbleeds (Kim et al. 2005;S S et al. 2008). On the other hand, it has been suggested that people with cardiovascular risk factors including hypertension, lacunar infarcts and white matter lesions may have more microbleeds in deep or infratentorial regions. This difference in distribution may indicate a difference in the etiology, lobar microbleeds being related to CAA (Greenberg et al. 1996) and deep ones being related to hypertensive or atherosclerotic microangiopathy (Vernooij et al. 2008b).

1.3: Histopathology of Brain Microbleeds

Histologically, two processes have been associated with the development of microbleeds as a result of the damage to the vessel walls of small- and medium-sized arteries, namely fibrohyalinosis and amyloid deposition (Fazekas et al. 1999). Microbleeds could be produced by gradual extravasations of erythrocytes through damaged vessel walls in the brain (Roob & Fazekas 2000) or by small areas of frank hemorrhage (Werring 2007).

Fazekas and colleagues correlated the areas of signal loss on GRE T2*W MRI with histopathology specimens in 11 patients. They confirmed that microbleeds are infact a focal collection of hemosiderin laden macrophages in 21 of 34 areas with signal loss on MRI (Figure 1.4 and 1.5). These deposits were frequently present in the basal ganglia and thalami, in the brains with fibrohyalinosis (adjacent to small blood vessels), but other foci in a corticosubcortical distribution were also noticed in two patients. MRI-negative hemosiderin deposits were also observed, and were smaller in size with only a few perivascular hemosiderin laden macrophages. No calcification or vascular malformations (e.g., cavernous hemangioma) were noticed as underlying MRI-defined microbleeds (Fazekas et al. 1999). They did not compare the size on histopathological specimens with the size on MRI. In reality, most microbleeds probably have a sub-millimeter size but the hemosiderin in them has a susceptibility effect on the local magnetic field leading to a larger area of signal loss on MRI images, of the order of millimeters (Viswanathan & Chabriat 2006).

In another study, hypointense lesions on GRE T2*W MRI were also found to be associated with ruptured arteriosclerotic vessels (< 200 μm in diameter), in 3 brain specimens on autopsy. They also noticed gliosis, ischemic necrosis and perivascular hemosiderin deposits in the surrounding areas in these specimens (Tanaka et al. 1999).

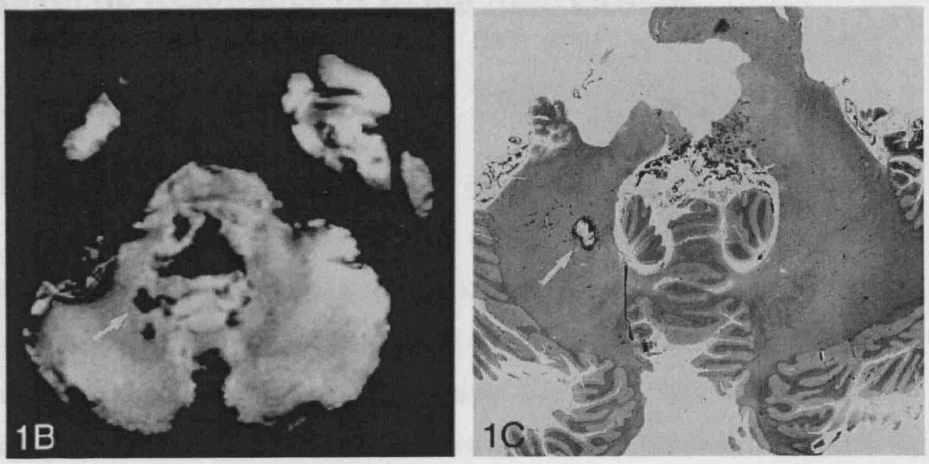


Figure 1.4: Comparison of brain microbleeds on GRE T2*w MRI with histopathological sections

Post-mortem gradient-echo T2*-weighted MR images showing foci signal loss in the cerebellum and pons (arrow B). C: Histopathological section showing an old microbleed (arrow), corresponding to the largest hypointensity (corresponding arrows in B and C). (Fazekas et al. 1999)

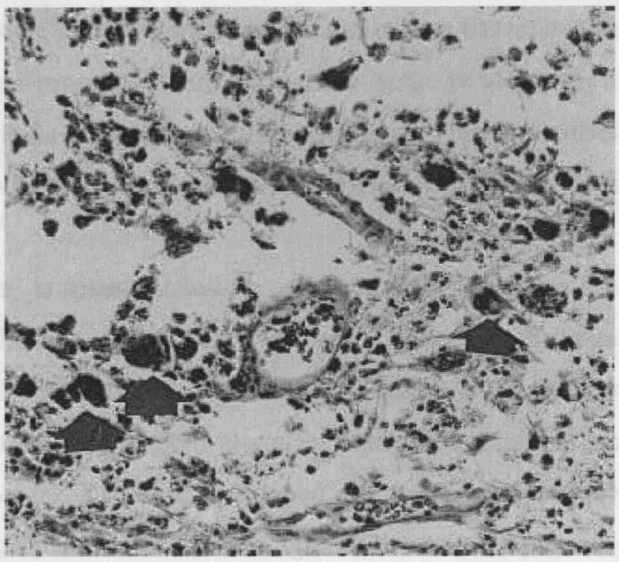


Figure 1.5: Histopathological appearance of brain microbleeds

MR-positive microbleed (2 to 3 mm) in the subcortical white matter. Numerous darkly stained hemosiderophages are seen in the centre, close to a ruptured vessel (arrows) (Fazekas et al. 1999)

1.4: Magnetic Resonance of Brain Microbleeds

1.4.1: Basic Principles

MRI is based on nuclear magnetic resonance (MR) principles and does not involve ionizing radiation. It creates images utilizing certain extrinsic scanning parameters [echo time (TE), repetition time (TR), field of view, slice thickness, slice gap and resolution], which are set by the physician or technologist, and the intrinsic (physical and chemical) properties of atoms within tissues (including spin angular moment, local magnetic field, spin-lattice relaxation and spin-spin relaxation). The scanning parameters are briefly defined below (Jackson et al. 1997;Tress & Brant-Zawadski 1985).

All tissues are composed of atoms. The nuclei of these atoms contain protons, and those with an odd nuclear number (protons + neutrons = odd number) have two important intrinsic properties; spin angular moment and a local magnetic field. The most important such atom in MRI is hydrogen nucleus (proton), contained in water molecules. In a resting state there is no net magnetization in a tissue, because the sum of all the spin vectors of protons is zero. When the tissue is placed in an external magnetic field these protons absorb or emit the energy and realign themselves, and a net magnetization is produced along the longitudinal axis of the magnetic field.

This net magnetization is manipulated to produce MR images. The simplest manipulation is the application of short burst of energy, called a radiofrequency (RF) pulse (usually this is a "90° pulse"). When the RF pulse is applied the protons undergo re-arrangement and are aligned along the transverse axis, perpendicular to the magnetic field. After the RF pulse is stopped, the protons release the absorbed energy and immediately try to return to their original orientation, a process known as relaxation (Brown & Semelka 2003). TE is the time between the application of the 90° pulse and the peak of the detected signal. TR is the time between consecutive application of the 90° pulses (Jackson et al. 1997;Tress & Brant-Zawadski 1985).

1.4.1a: Spin-Lattice Relaxation (T1) Time and Spin-Spin Relaxation (T2) Time

After the application of RF the longitudinal magnetization is lost, and the magnetization of the tissue is in the transverse plane. The time required for protons to achieve longitudinal magnetization again (63% of its original value) by releasing the absorbed energy is called longitudinal relaxation (T1). The released energy is transferred to its surroundings; therefore, it is also called spin-lattice relaxation time. In comparison, the time required for the transverse magnetization to decay to 37% of its initial value is called transverse relaxation (T2). The released energy is transferred from an excited proton to a neighboring proton; therefore, it is called spin-spin relaxation. T2 is always less than or equal to T1 (Brown & Semelka 2003).

1.4.1b: T2* Effect

An important aspect of MR is that the magnetic field applied is never 100% homogeneous. This inhomogeneity may come from the main scanner magnetic field inhomogeneity (due to near by building walls and metals) and tissue-induced non-uniformity. When a tissue is placed in a magnetic field, its different parts behave differently because of their intrinsic paramagnetic properties. This magnetic effect on the local magnetic field is called magnetic susceptibility. Interfaces between soft tissues, bone and air (which have different magnetic susceptibility properties) also make the applied magnetic field inhomogeneous (Roberts & Mikulis 2007). The problem of susceptibility can be solved by applying a second redirecting or refocusing RF pulse (Brown & Semelka 2003). In cases where the second redirecting RF pulse is not applied, ongoing dephasing of the protons occurs and their vectors remain incompletely aligned. This results in low signal on MRI (Brown & Semelka 2003;Rajan 1998).

Certain tissues, e.g. blood break down products (hemosiderin, deoxyhemoglobin and methemoglobin) have paramagnetic properties (positive magnetic susceptibility) and when they are imaged by MRI, augmented spin dephasing and signal loss is observed. This effect causes these tissues to appear as large area of signal loss on MR images than the original size of the paramagnetic material, and is called the

blooming or T2* effect (Koennecke 2006;Roberts & Mikulis 2007;Viswanathan & Chabriat 2006). When imaging microbleeds, this T2* effect is critical, since they are only seen due to their susceptibility effect.

1.4.1c: Pulse Sequences: Spin Echo and Gradient Recall Echo

A pulse sequence defines the manner in which the radiofrequency pulses and magnetic gradient fields are applied (Jackson et al. 1997;Scherzinger & Hendee 1985). The SE is the most commonly applied sequence in neuroradiology. It has two user selected delays TE and TR and two RF pulses. The 90° pulse creates the detectable magnetization, and the180° pulse refocuses it at the TE (Jackson et al. 1997;Scherzinger & Hendee 1985).

The GRE is another MRI sequence which is significantly faster than SE sequence. It is different from SE as there is no refocusing RF pulse (180° pulse), and the single RF pulse is less than 90°. Due to the absence of redirecting RF pulse, the susceptibility-related signal loss increases because of the field inhomogeniety, i.e. the T2* effect is emphasized, as discussed earlier. Thus, this sequence positively exploits the magnetic field inhomogeneities. This type of imaging protocol is termed a T2*W GRE sequence (Brown & Semelka 2003;Jackson et al. 1997;Rajan 1998;Scherzinger & Hendee 1985). This sequence is used in a variety of clinical implications where paramagnetic blood breakdown products are to be detected (Jackson et al. 1997;Roberts & Mikulis 2007;Scherzinger & Hendee 1985).

1.4.2: MRI sequences and Brain Microbleeds

Although Scharf used conventional SE sequences to detect hemorrhagic lacunes in 1994 (Scharf et al. 1994), the lesions then detected are probably not the same as microbleeds as defined today. Later, Offenbacher used GRE sequences to detect microbleeds as it has a greater sensitivity than SE for the detection of hemosiderin deposits (Offenbacher et al. 1996). GRE is now the most widely used sequence for microbleed imaging.

As discussed above, microbleeds also appear larger on GRE sequences than the actual tissue lesions because of the "blooming effect." GRE sequences can detect both present and past microbleeds as hemosiderin in haemorrhage is believed to remain in macrophages for many years or indefinitely after hemorrhage (Koennecke 2006;Viswanathan & Chabriat 2006). Thus the age of microbleeds cannot be determined on GRE images.

The strength of the magnetic field is an important factor influencing the appearance of microbleeds on T2*W GRE. GRE sequences at 3 T were found to be more sensitive than 1.5 T for the detection of microbleeds (Figure 1.6) (Scheid et al. 2007). TE may be another important parameter for the detection of microbleeds. Theoretically, the signal loss increases on T2*W GRE sequences by lengthening TE and decreasing the flip angle (Jackson et al. 1997;Roberts & Mikulis 2007;Scherzinger & Hendee 1985). Tatsumi documented in a case report that the number and size of microbleeds changed considerably when TE was prolonged from 10ms to 23ms (Figure 1.7) (Tatsumi et al. 2008). This observation needs to be further evaluated in a larger population.

Susceptibility weighted imaging (SWI) is a new technique, which also utilizes the magnetic properties of tissues. It creates magnitude or phase images, or a combination of both, and these images are three-dimensional (3-D) and velocity-compensated. Small veins and microbleeds become increasingly visible with SWI, and it also detects microbleeds not identified on T2*W GRE. Unwanted effects of T2*W GRE e.g. background field variations (air–tissue interfaces) are less on SWI (Sehgal et al. 2005). Furthermore, SWI can also differentiate between diamagnetic (calcium) and paramagnetic (blood) susceptibility effects (Figure 1.8) (Thomas et al. 2008;Vernooij et al. 2008a). However, SWI may detect more artefacts in addition to microbleeds, e.g. blood vessels, so it requires further investigation. It is likely that SWI will become increasingly used in microbleed imaging studies. Recently, diffusion weighted imaging (DWI) was used to detect microbleeds in a patient with acute ischemic stroke (Taguchi et al. 2007).

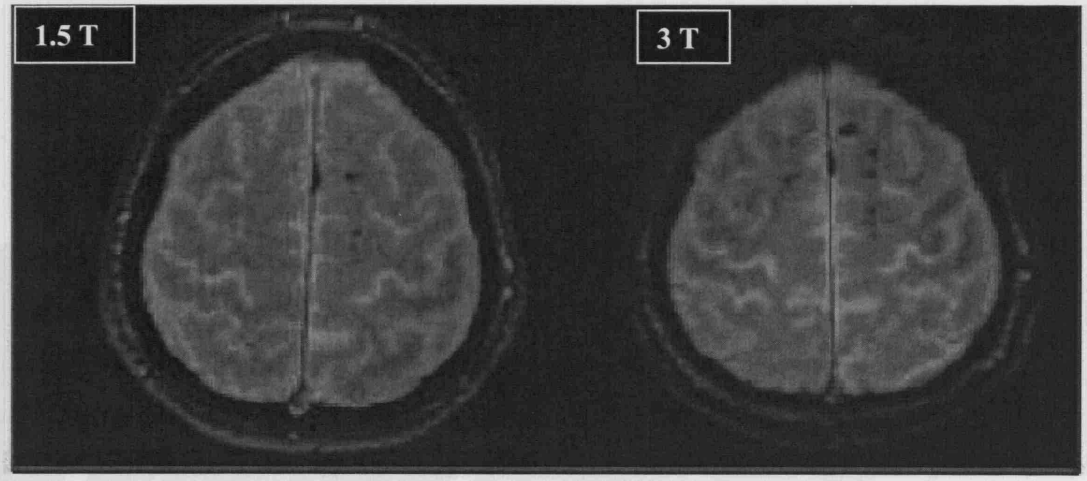


Figure 1.6: Brain microbleeds on 3T GRE MRI and 1.5T GRE MRI

Increase in number of microbleeds seen on 3T GRE MRI as compared to 1.5 T GRE MRI
(Scheid et al. 2007)

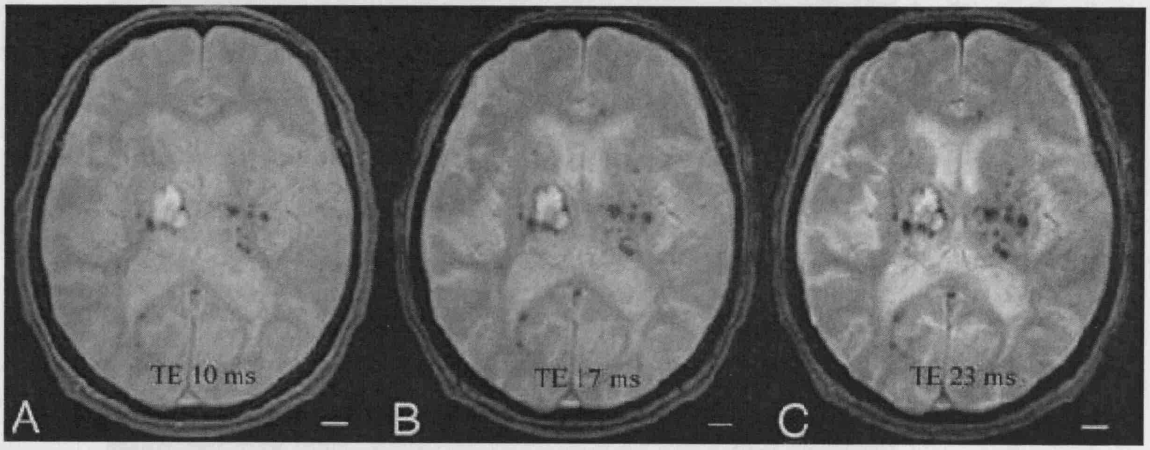


Figure 1.7: Increase in number and size of brain microbleeds by increasing TE from A to C
(Tatsumi et al. 2008)

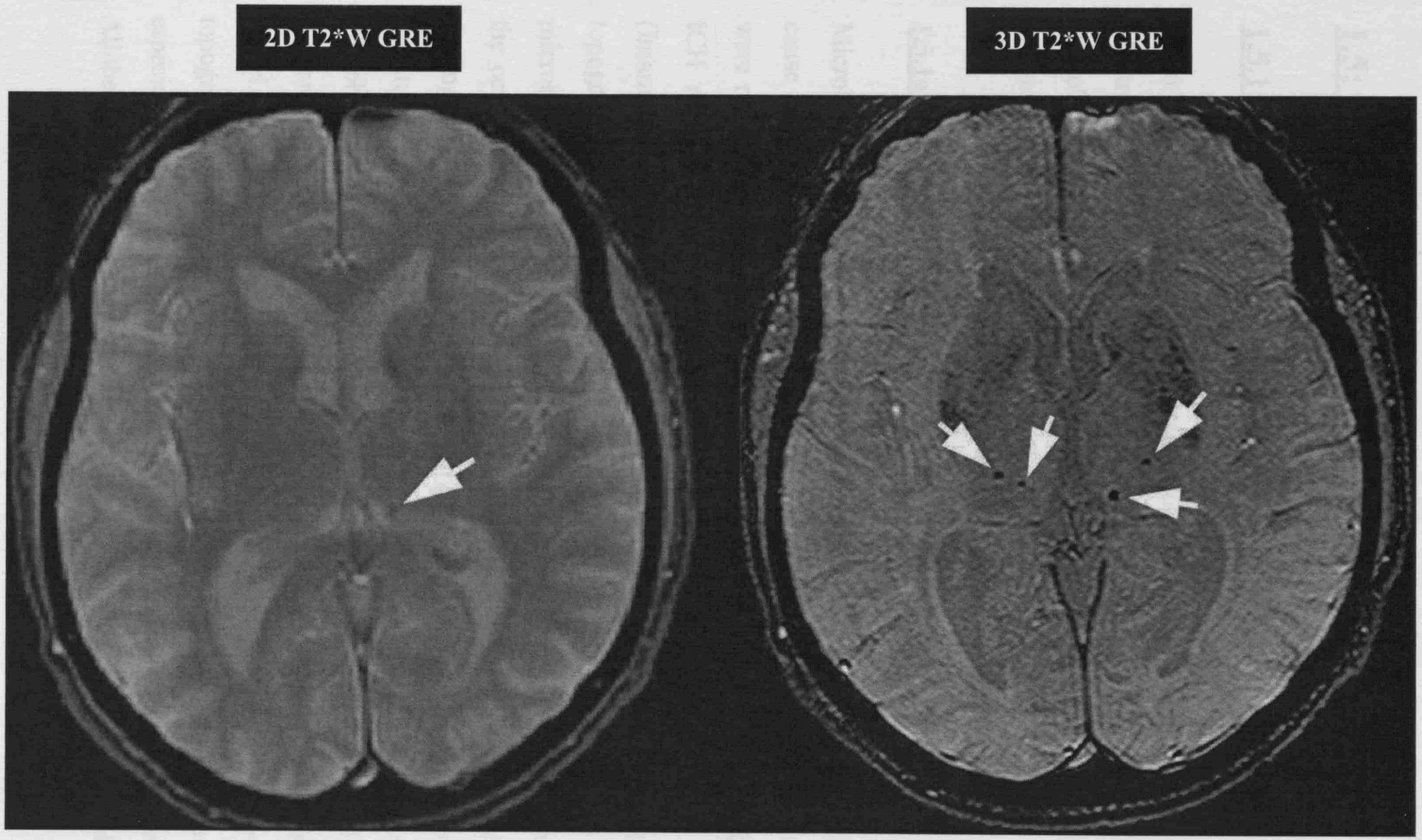


Figure 1.8: Brain microbleeds on accelerated 3D T2*W GRE and conventional 2D T2*W GRE

Change in appearance and number of microbleeds on accelerated 3D T2*-weighted GRE as compared to conventional 2D T2*-weighted GRE (Vernooij et al. 2008a)

1.5: Clinical Implications of Brain Microbleeds

1.5.1: Brain Microbleeds and Stroke

Brain microbleeds are a risk factor for stroke and are present in all different subtypes of stroke. It is reported that non-traumatic ICH has the highest frequency of microbleeds followed by lacunar infarction, cardio-embolic infarction and atherothrombotic infarction (Kato et al. 2002).

1.5.1a: Brain Microbleeds in Intracerebral Hemorrhage (ICH)

Microbleeds are strongly associated with ICH, but whether they are an important cause remains to be definitely established. In a recent systematic review microbleeds were reported in 60% of patients with ICH (Cordonnier et al. 2007). The volume of ICH may be more than two to threefold higher in patients with microbleeds (Imaizumi et al. 2008;Lee et al. 2006). Microbleeds also correspond to the topographic distribution of ICH, and new ICHs may arise in the areas where microbleeds were present. Lee described a significant predilection of microbleeds for the central portion of the pons and the dentate nucleus of the cerebellum. These findings correlate with the topography of infratentorial ICH described in the literature (Lee et al. 2004d). In another study of supratentorial ICH Lee reported that microbleeds show a significant predilection for the temporo-occipital area of the subcortical white matter, the posterolateral part of the upper putamen, and the lateral nuclei of the mid-level thalamus, which is quite similar to the supratentorial topography of ICH (Lee et al. 2004c). Patients on maintenance hemodialysis, especially those with ICH, had a higher frequency of microbleeds (Watanabe 2007). All these observations suggest that microbleeds are probably causally linked to ICH.

1.5.1b: Brain Microbeeds in Ischemic Stroke, Transient Ischemic Attacks and Leukoaraiosis

Microbleeds are seen in 34% of patients with ischemic stroke, and of ischemic strokes, lacunar infarctions have been found to have the highest prevalence of microbleeds in a systematic review (Cordonnier et al. 2007). The involvement of microbleeds with lacunar stroke may signify a shared underlying small vessel disease (SVD) (Cordonnier et al. 2007;Fan et al. 2004). One study showed that microbleeds are twice more frequent in lacunar infarctions than cortical strokes (Wardlaw et al. 2006). Another study reported that patients with microbleeds and no advanced leukoaraiosis develop ICH alone, and patients with microbleeds and leukoaraiosis develop ischemic strokes (Naka et al. 2006).

Werring compared microbleeds is patients with transient ischemic attacks (TIA) and ischemic stroke. They found that only 2% of patients with TIA had microbleeds as compared to 33% of patients with ischemic strokes (Werring et al. 2005).

Microbleeds have also been found to be associated with leukoaraiosis (Nighoghossian et al. 2002;Roob et al. 2000), supporting the idea that they may be a marker of vasculopathy and may also be linked to poor collateral supply and increased likelihood of persistent cerebrovascular symptoms. A recent paper reported that leukoaraiosis was associated with more severe infarct growth in acute ischemic stroke, which is also consistent with this idea (Ay et al. 2008).

1.5.1c: Brain Microbleeds in Recurrent Stroke

Recurrent strokes, whether hemorrhagic or ischemic, have higher prevalence of microbleeds (Cordonnier et al. 2007). Recurrent ICH alone (80-90%) was found to have the highest prevalence of microbleeds as compared to combination of ICH and ischemic stroke (76.5%) or ischemic stroke alone (50.0%) (Cordonnier et al. 2007;Naka et al. 2004). Fan described that old microbleeds in patients with ischemic stroke may predict subsequent cerebral bleeding, but the number of outcome events was small (Fan et al. 2003). Other studies have also reported that they may predict

the recurrence of stroke (lacunar stroke and ICH) in those patients with a pre-existent lacunar infarction or ICH (Imaizumi et al. 2004). Thus, microbleeds may be helpful for the future prediction of ICH, if seen on initial MR imaging in patients with primary ICH (Greenberg et al. 2004;Jeon et al. 2007), hypertension and advanced leukoaraiosis (Lee et al. 2005).

1.5.2: Brain Microbleeds: Cerebral Amyloid Angiopathy and Hypertensive Angiopathy

CAA is an important cause of ICH and is suggested when recurrent hemorrhages are present in a lobar distribution (Knudsen et al. 2001). Small and medium sized vessel and cortical cerebral vessels are affected by the amyloid deposition in their walls which makes them weak and fragile. Greenberg reported petechial hemorrhages in cortical or corticosubcortical regions in 80% of patients of CAA, who also had lobar intracerebral hemorrhages. These findings suggest that CAA might be neuroradiologically diagnosed and staged during life (Greenberg et al. 1996). On the other hand it is hypothesized that microbleeds in deeper regions like basal ganglia, brainstem and cerebellum may reflect hypertensive SVD (Vernooij et al. 2008b;Werring 2007). Some studies show that, patients with ICH in basal ganglia had microbleeds in basal ganglia and other deeper structures (Lee et al. 2004b;Roob et al. 2000). Further studies are required to confirm whether the topographic distribution of microbleeds in patients with CAA and hypertensive SVD has any clinically useful diagnostic significance. A challenge for designing studies to answer this question is that CAA and hypertensive arteriopathy may often co-exist.

1.5.3: Other Cerebrovascular Disorders and Brain Microbleeds

In small studies other CVDs including **c**erebral **a**utosomal **d**ominant **a**rteriopathy with **s**ubcortical **i**nfarcts and **l**eukoencephalopathy (CADASIL) (Lesnik Oberstein et al. 2001), Binswanger's disease (Hanyu et al. 2003b) and Moya moya disease (Kikuta et al. 2005) have been shown to have more microbleeds than the normal population. In CADASIL the risk is independent of hypertension but is associated with the Arg153Cys mutation, indicating that these patients may have an increased

risk for ICH or bleeding prone angiopathy. This finding may have important repercussions in deciding patient management (Dichgans et al. 2002;Lesnik Oberstein et al. 2001). In another small study of 63 patients Kikuta reported that 44% of patients with Moya moya disease had microbleeds (Kikuta et al. 2005).

1.5.4: Do Brain Microbleeds cause Neurological Symptoms?

1.5.4a: Cognitive Disorders

Microbleeds have a high prevalence in patients with a range of cognitive disorders. In a study of 772 patients with memory problems 17% had at least one microbleed. Sixty-five percent of vascular dementia (VD) patients, 18% of Alzheimer disease (AD) patients, 20% of mild cognitive impairment patients and 10% of patients with subjective complaints were noticed to have microbleeds (Cordonnier et al. 2006). They are reported to have a predilection for the temporoparietal area in clinically diagnosed VD (Won et al. 2007). In another study on AD patients, microbleeds have been documented whether these patients had associated CVD (12.5%) or did not have associated CVD (16.7%) (Nakata-Kudo et al. 2006). Werring evaluated cognitive impairment in patients with microbleeds. They were observed to be associated especially with executive dysfunction, which was noticed in 60% of microbleed positive patients compared with 30% of carefully matched non-microbleed control patients. Furthermore, those patients with frontal-executive dysfunction had more microbleeds in the frontal regions (Figure 1.9) and in the basal ganglia, suggesting that their cognitive effect may be mediated by the disruption of strategic fronto-subcortical circuits (Werring et al. 2004).

1.5.4b: Clinical Syndromes

Initially microbleeds were considered to be clinically silent; Kwa and colleagues argued that in 24 patients with microbleeds out of 31, microbleeds were asymptomatic (Kwa et al. 1998). There are individual case reports in which patients have shown clinical features, which can be attributed to the location of microbleeds, without any alternative explanation. Watanabe described a case of 72 year old man

who developed voluntary left lateral gaze disturbance secondary to the development of a new microbleed in right medial leminiscus (Figure 1.10) (Watanabe & Kobashi 2005). Patients with CAA are increasingly recognized to present with focal neurological disturbances that are atypical for both TIA and partial seizures.

1.5.5: Antithrombotic / Thrombolytic Treatment and Brain Microbleeds

As a potential risk factor for future development of ICH, microbleeds may have a role in deciding the antithrombotic and thrombolytic management plan for patients with stroke. This hypothesis has been investigated for the administration of thrombolytic therapy in acute stroke and long term aspirin administration. Previously, it was reported that silent microbleeds may be a marker of increased risk of hemorrhagic transformation (HT) in patients receiving thrombolytic therapy for acute ischemic stroke (Kidwell et al. 2002). However, recent studies suggest that the presence of microbleeds is not associated with increased risk of HT after thrombolytic treatment (Fiehler et al. 2007;Kakuda et al. 2005;Kim et al. 2006). Fiehler suggested that the increased risk of symptomatic ICH secondary to microbleeds is small and not likely to exceed the benefits of thrombolytic therapy. However, patients with many microbleeds may be at increased risk (Fiehler et al. 2007). In a case-control study microbleeds were found to be associated with aspirin related ICH (Wong et al. 2003), though there was no non- aspirin related ICH control group in this study. Further case-control or prospective studies including comparison with non-aspirin related ICH are thus required to explore this important question further.

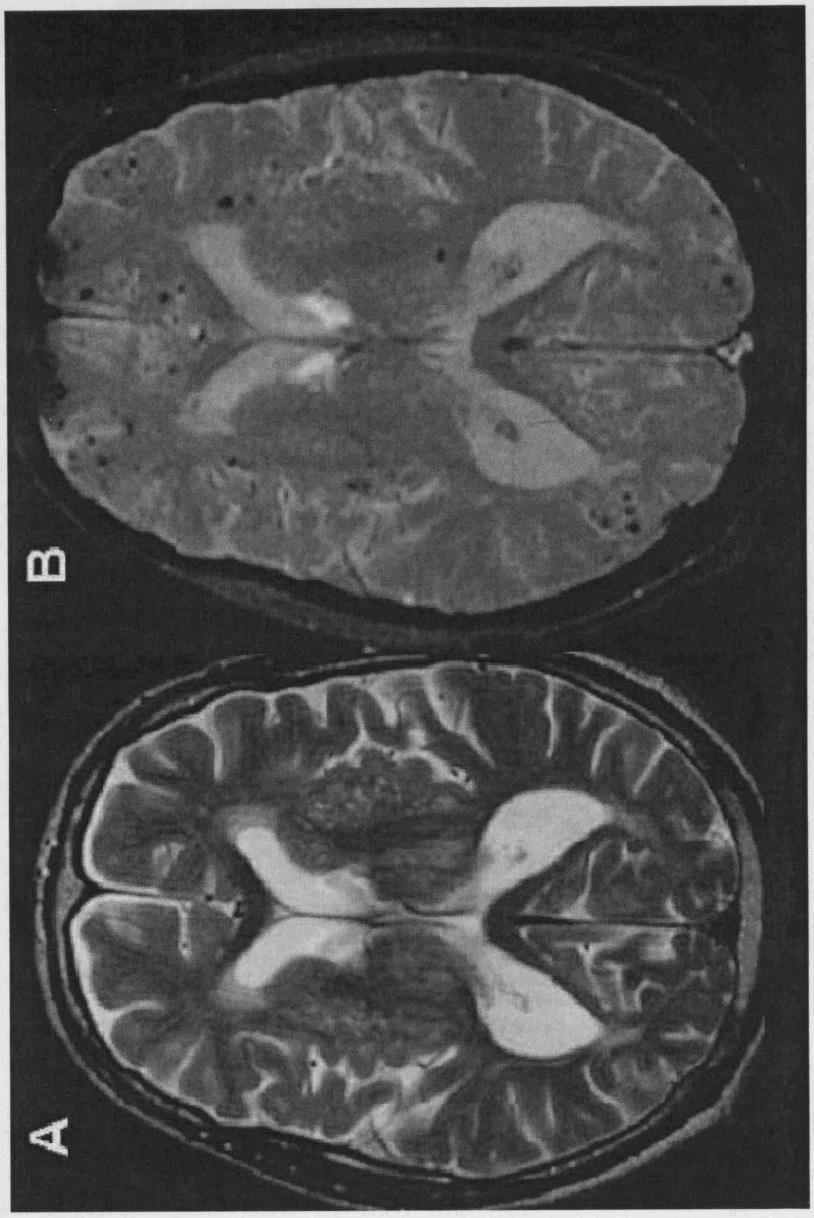


Figure 1.9: Brain microbleeds associated with executive dysfunction

Multiple microbleeds in frontal lobes on T2*W GRE MRI in a patient with executive dysfunction (Werring et al. 2004)

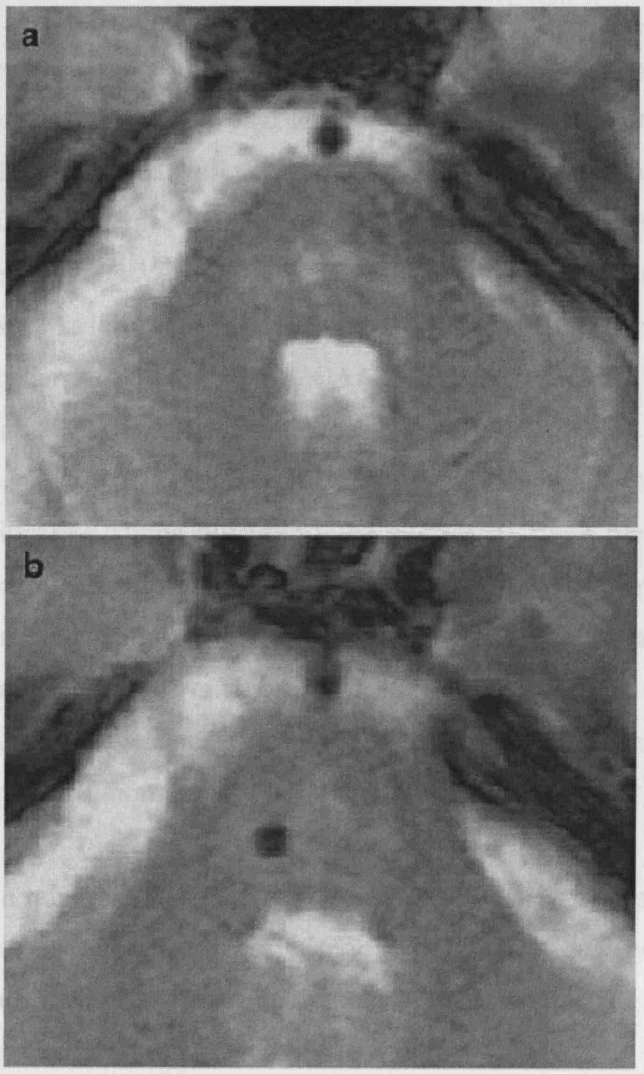


Figure 1.10: Clinical symptoms produced by brain microbleeds

a) No microbleeds in brainstem, prior to symptoms
b) b) Development of a new microbleed causing lateral gaze disturbance
(Watanabe & Kobashi 2005)

1.6: Rationale and Purpose of the Project

In the past few years, research on microbleeds has expanded very rapidly. Multiple research papers have been published on prevalence, risk factors, predictive value and association of microbleeds with different diseases. The focus of available published literature is the prevalence and distribution of microbleeds in healthy population (Jeerakathil et al. 2004;Vernooij et al. 2008b) or their association with particular clinical diseases like stroke and its subtypes (Fiehler et al. 2007;Jeong et al. 2004;Lee et al. 2004d;Werring et al. 2005), CAA (Greenberg et al. 1996), CADASIL (Dichgans et al. 2002), Binswanger's disease (Hanyu et al. 2003b), vascular dementia and Alzheimer's disease (Hanyu et al. 2003a), as discussed earlier. However, a number of critical questions still need to be addressed. These questions include the important clinical implications already discussed e.g.; whether the presence of microbleeds should influence antithrombotic or thrombolytic treatment, and the possible diagnostic value of microbleeds in ICH, in particular in CAA. The role of microbleeds in the development of cognitive impairment in patients with dementia also needs further exploration.

The studies on microbleeds so far have not provided consistent conclusions. This is in part because there are no precise criteria to define and identify microbleeds, nor are there standardized MRI parameters (table 1). In addition, to answer the above mentioned questions regarding microbleeds a reliable rating scale is required, which can identify the presence and anatomical distribution of microbleeds with good intra and inter-rater agreement, applicable across different MRI sequence types. Few previous studies have clearly discussed the process of identification of microbleeds in their methods, and intra and inter-rater agreement for microbleeds has not been systematically reported in detail. The majority of the past studies calculated only the inter-rater agreement for the presence of microbleeds (table 2).

The purpose of this project is to systematically evaluate the intra and inter-rater agreement for the presence, number and anatomical distribution of brain microbleeds

using an anatomical rating scale, in a population representative of the spectrum of diagnoses seen in an acute stroke unit. We also want to test this rating scale on two different GRE MRI sequences (TE = 40msec, TE = 26 msec), by two observers/raters of varying levels of experience in MRI microbleed research.

Table 1: Difference in MRI Specifications and Size of Brain Microbleeds in Previous Studies

Authors	Time Echo (TE) msec	Time Repeat (TR) msec	Magnet (Tesla)	Slice Thick-ness mm	Slice Gap mm	Size of Microbleeds mm
(Cordonnier et al. 2006)	22	800	1	5	1.5	<10
(Dichgans et al. 2002)	22	1000	1.5	4	0	>2
(Fan et al. 2004)	30	350	1.5	5	0.5	-
(Fan et al. 2003)	30	300	1.5	5	0.5	-
(Fazekas et al. 1999)	15	550-650	1.5	5	0.5	5
(Fiehler et al. 2007)	14-49	0.8-2140	-	5 to 7	0 to 2	<5
(Greenberg et al. 2004)	50	750	1.5	-	-	-
(Hanyu et al. 2003b)	20	500	1	8	2	>2
(Henskens et al. 2008)	23	736	1.5	5	0.5	<5
(Imaizumi et al. 2004)	26	450	1.5	8	No gap	Up to 7
(Imaizumi et al. 2008)	26	450	1.5	8	No gap	Up to 7
(Jeerakathil et al. 2004)	26	760	1	5	0.5	<10
(Jeon et al. 2007)	30	430	1.5	5	2	<5
(Jeong et al. 2004)	15	200-500	1.5	1.4	0.7	5
(Kakuda et al. 2005)	14-47	450-800	1.5	5	2.5	5
(Kato et al. 2002)	26	800	1.5	5	1.5	-
(Kidwell et al. 2002)	15	800	1.5	7	No gap	<5
(Kikuta et al. 2005)	18	612	3.5	5	1.5	<10
	17	700	1.5	5	1	<10
(Kim et al. 2005)	-	-	-	-	-	Up to 5
(Kim et al. 2006)	30	400	-	5	2	5
(Kwa et al. 1998)	-	-	1.5	-	-	-
(Lee et al. 2004b)	15	200-500	1.5	5	2	5
(Lee et al. 2004a)	15	200-500	1.5	5	2	Up to 5
(Lee et al. 2005)	15	500	1.5	-	-	-
(Lee et al. 2006)	15	500	1.5	-	-	-
(Lee et al. 2007)	15	500	1.5	-	-	-
(Lee et al. 2004c)	15	500	1.5	6	2	5
(Lee et al. 2004d)	15	500	1.5	6	2	Up to 5
(Lemmens et al. 2007)	16/26/35	710-1000	1/1.5/3	7	-	<5
(Lesnik Oberstein et al. 2001)	48	2598	1.5	6	0.6	-
(Naka et al. 2006)	26	800	1	5	1.5	-
(Naka et al. 2004)	26	800	1	5	1.5	-
(Nakata-Kudo et al. 2006)	23	667	1.5	5	1	-
(Nighoghossian et al. 2002)	26	800	1.5	5	-	2 to 5
(Offenbacher et al. 1996)	15-20	500-720	1.5	-	-	2 to 5
(Roob et al. 2000)	16	600-800	1.5	5	10%	2 to 5
(Roob et al. 1999)	16-20	600-800	1.5	5	10%	2 to 5
(S S et al. 2008)	50	3050	1.5	3	Interleaved	-
(Sorimachi et al. 2007)	15	675	1	6	1	2 to 5
(Vernooij et al. 2008a)	20 31	775 45	1.5	-	-	<10
(Vernooij et al. 2008b)	31	45	1.5	-	-	<10
(Wardlaw et al. 2006)	15	625	1.5	5	1	<5
(Watanabe 2007)	18	600	1.5	5	1.5	<5
(Werring et al. 2005)	40	300	1.5	5*	1.5*	<10
(Werring et al. 2004)	40	300	1.5	5*	1.5*	<10
(Won et al. 2007)	-	-	-	-	-	<10
(Wong et al. 2003)	30	300	1.5	5	0.5	-

*Through personal Communication

Table 2: Intra and Inter-rater Agreement for Microbleeds in previous studies

Authors	Inter-rater Kappa (κ) Value	Intra-rater Kappa (κ) Value
(Fan et al. 2004)	κ = 0.75	
(Jeerakathil et al. 2004)	κ = 0.33 to 0.57 (Presence of Microbleeds)	
(S S et al. 2008)	κ = 0.71 to 0.73 (Presence of Microbleeds)	κ = 1.0
(Lee et al. 2007)	κ = 0.86	
(Lee et al. 2005)	κ = 0.87	
(Kwa et al. 1998)	κ = 0.6	
(Vernooij et al. 2008b)	κ = 0.85 (Presence of Microbleeds)	κ = 0.87
(Cordonnier et al. 2008)	κ = 0.68 (all locations) κ = 0.78 (lobar locations)	
(Vernooij et al. 2008a)	κ (2DT2*W GRE) = 0.80 κ (3DT2*W GRE) = 0.85 (Presence of Microbleeds) κ (2DT2*W GRE) = 0.90 κ (3DT2*W GRE) = 0.82 (Number of Microbleeds)	

Chapter 2: Materials and Methods

2.1: Subjects

The initial cohort consisted of 360 consecutive unselected subjects, presenting with suspected stroke to Acute Brain Injury Unit, National Hospital for Neurology and Neurosurgery (NHNN) from July 2004 to October 2007, and had T2*W GRE MRI. Fifty seven subjects were excluded because of the movement artifact on MRI scans, unavailable T2*W GRE MRI on Picture Archival and Communications System (PACS), missing MRI slices and TE = 15 msec (Figure 2.1). The final cohort consisted of 303 patients. These patients were divided into two groups, 273 patients with TE = 40 msec and 30 patients with TE = 26 msec for the T2*W GRE MRI. The study was approved by the NHNN and Institute of Neurology Joint Research Ethics Committee.

2.2: MRI specifications

All the subjects had MR imaging according to the stroke protocol followed at NHNN, and were carried out on 1.5 Tesla scanners. MRI scans with TE = 40 msec for T2*W GRE MRI sequences were done on a General Electric Medical Systems scanner (Genesis-Signa). MRI scans with TE = 26 msec for T2*W GRE MRI sequences were done on a Seimens scanner (Avanto). MRI parameters for the T2W FSE and GRE sequences used are shown in table 3.

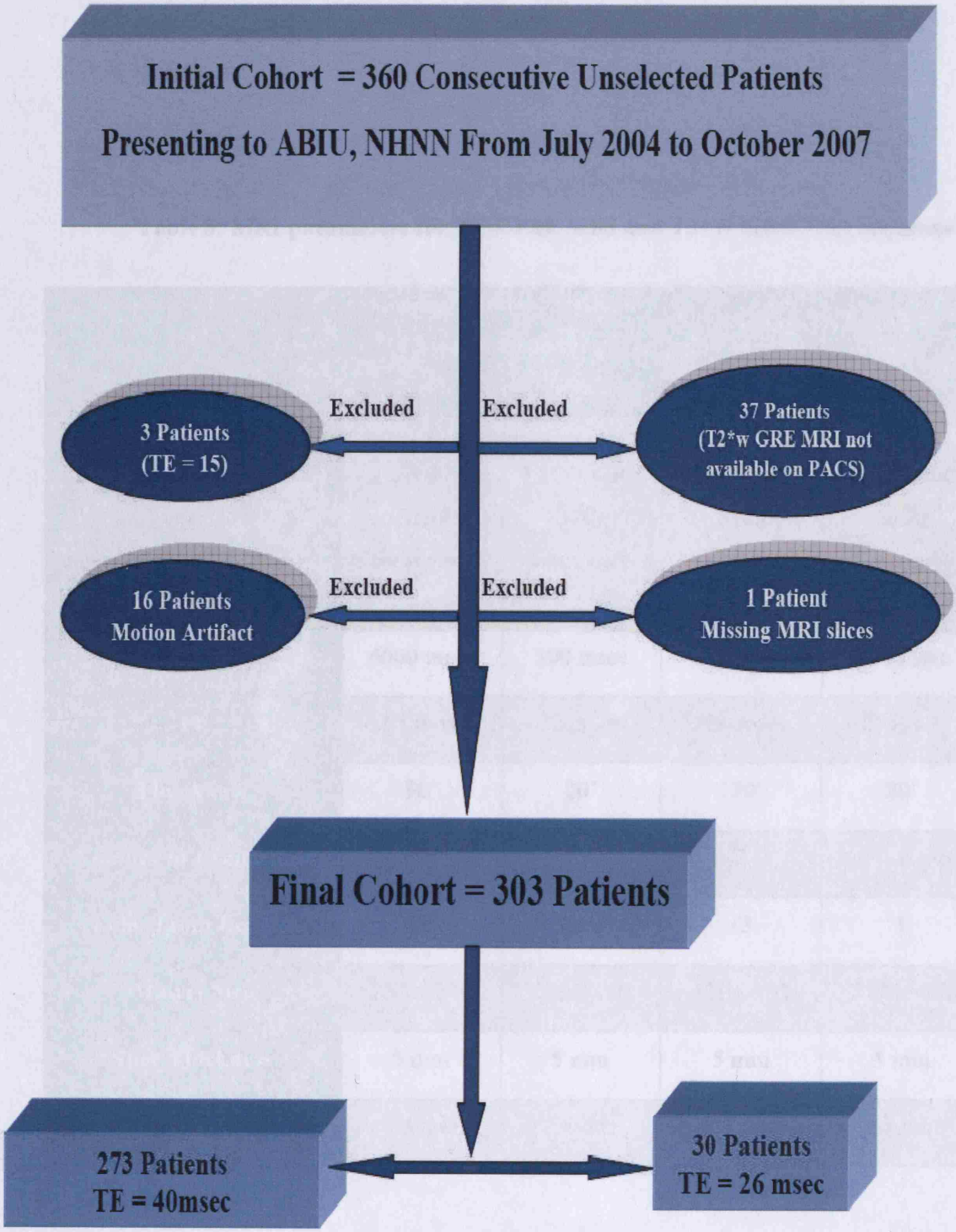


Figure 2.1: Flowsheet Diagram of Patient Cohort

Table 3: MRI parameters for T2W FSE MRI and T2*W GRE MRI sequences

Parameter	General Electric Medical Systems scanner (Genesis_Signa)		Seimens Scanner (Avanto)	
	T2W FSE MRI Sequence	T2*W GRE MRI Sequence	T2W FSE MRI Sequence	T2*W GRE MRI Sequence
TR	6000 msec	300 msec	4320 msec	800 msec
TE	105 m sec	40 m sec	106 msec	26 msec
Flip Angle	90˚	20˚	150˚	20˚
Number of Excitations	2	1	2	1
Echo Train Length	20	Zero	13	1
Matrix Size	256 x 224	256 x 160	448 x 392	512 x 448
Slice Thickness	5 mm	5 mm	5 mm	5 mm
Slice Gap	1.5 mm	1.5 mm	1.5 mm	1.5 mm

2.3: Image Analysis

Image analysis was performed by two independent raters, blinded to the medical history and clinical findings. Rater 1 (SG) is a neurologist with over 1 year experience in rating microbleeds. Rater 2 (UJC) is a training neurologist with one month experience in rating microbleeds. Both the raters received formal training by a consultant stroke neurologist (DW) and a consultant neuroradiologist (RJ) for the identification of microbleeds and their differentiation from other mimics. They were also trained in neuroanatomy and neuroradiology to clarify the anatomic boundaries before starting the image analysis. Rater 1 analyzed the MRI scans once, and rater 2 analyzed the MRI scans twice, with a gap of 4 weeks between the first and the second rating. Radiological diagnosis of ICH, ischemic infarcts, SVD, and other diagnosis were categorized from neuroradiologist reports. Ischemic infarcts included acute infarcts, old infarcts and lacunar infarcts.

2.4: Definition of Brain Microbleeds and Mimics

Brain microbleeds were divided into two categories; "definite" and "possible", since in some cases, the rater is not certain that a given lesion is a microbleed or a mimic (see below). A pilot study has suggested that this approach improves inter-rater agreement (Cordonnier et al. 2008). Definite microbleeds were defined as small, well defined, rounded/circular and dot-like hypointense lesions with clear margins on T2*W GRE MRI, ranging from 2 to 10 mm in size. We took an upper size limit of 10 mm as this was the largest cut off size reported in the previous studies (table 1), and we wanted the project to be as inclusive as possible. Possible microbleeds were identified as less hypointense lesions as compared to definite ones, without well defined or clear margins but of round/circular shape.

Two hypointensities at the same place in two successive slices of T2*W GRE MRI were considered as a single microbleed. Any linear lesion which was either deep or superficial, hypointense structures in subarachnoid spaces, symmetrical hypointensities in the globi pallidi (likely to be calcification or iron deposition) or dentate nuclei, flow voids in the subarachnoid space from cortical vessels and signal

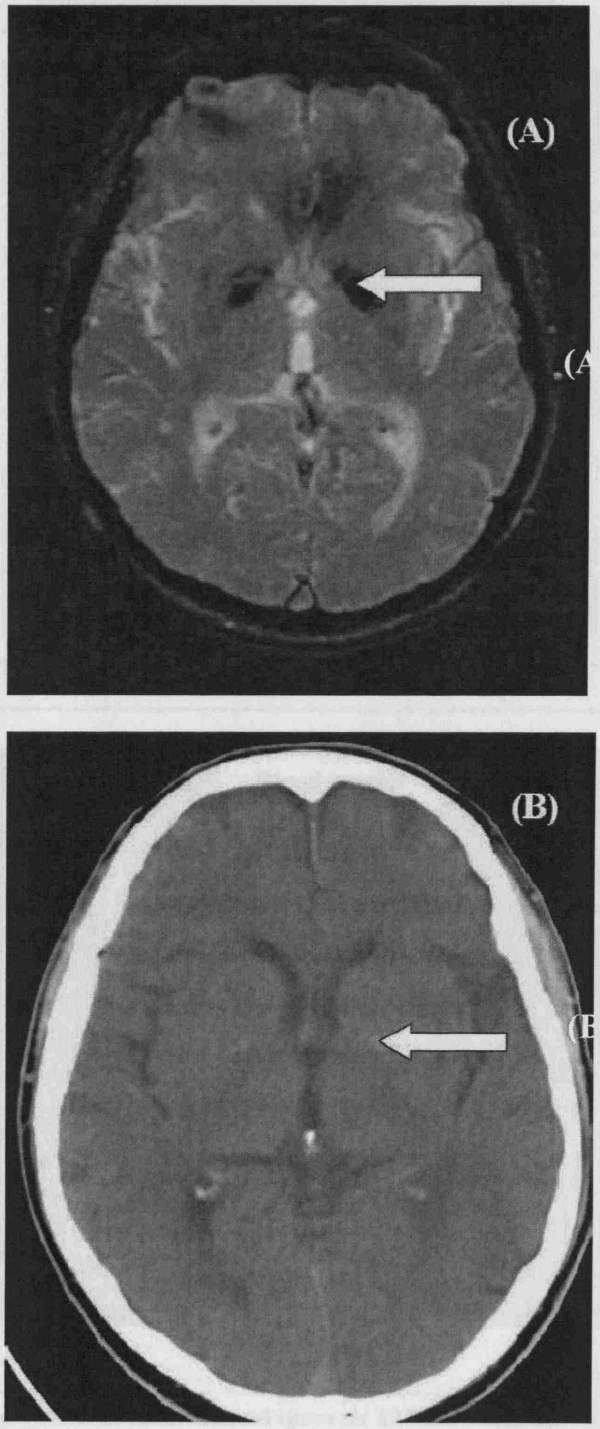
loss from air-bone interfaces were carefully excluded (Figure 2.2). Haemorrhage within an area of infarction (haemorrhagic transformation) and small circular haemorrhages in close proximity to or adjacent to a primary ICH or an infarct were also excluded. Mimics of microbleeds were differentiated from microbleeds using CT (Computed Tomography) scans (for visualizing calcification) and Fluid Attenuated Inversion Recovery (FLAIR) MRI (for visualizing subtle areas of infarction or detailed coronal anatomy), where available.

2.5: Anatomical Boundaries

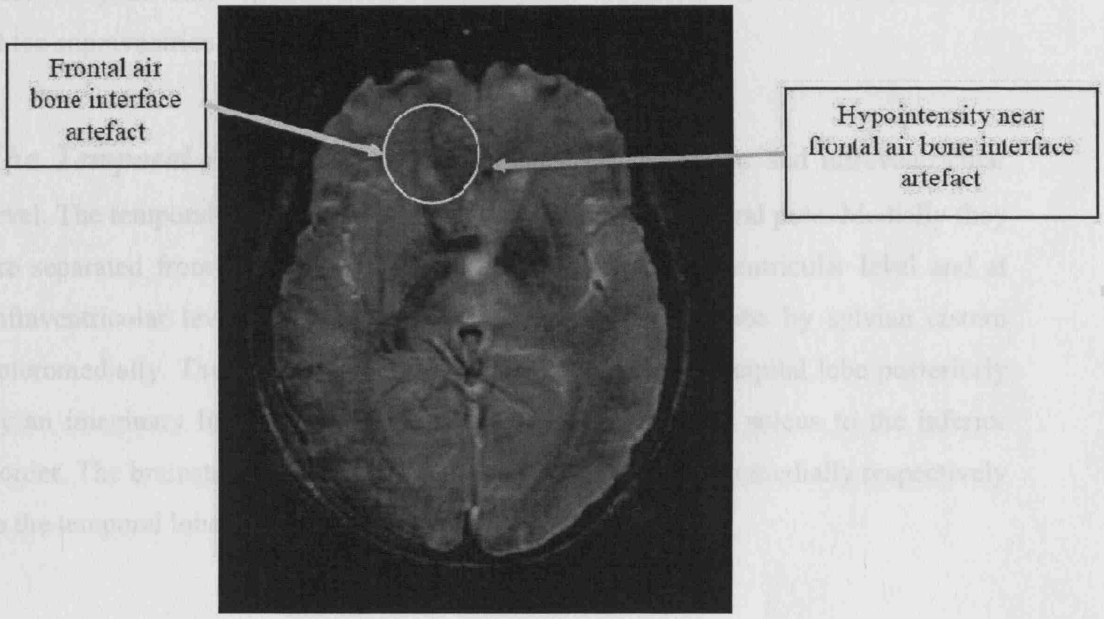
Both definite and possible microbleeds were counted in right and left cerebral hemispheres. Each cerebral hemisphere was categorized into lobar, deep and infratentorial regions. The lobar category included frontal, parietal, temporal and occipital lobes. The deep category included both the deep white and the grey matter. Microbleeds in this category were divided into the following locations; Thalamus, Lentiform, Caudate, Internal capsule, External capsule, Insula, Corpus Callosum and Centrum Semiovale. The infratentorial category included the brainstem and the cerebellum. The number and distribution of microbleeds were recorded on a predesigned rating scale shown in (Figure 2.3). The cerebral lobes, deep grey and white matter, brainstem and cerebellum were identified on axial and sagittal sections of MRI scans according to the definitions and characteristic features as described by Stark and Bradley (Stark & Bradley 1999). The anatomical regions are illustrated in Fig 2.4 and are defined in detail below.

The Frontal Lobes lie between frontal pole anteriorly, central sulcus posteriorly, interhemispheric fissure medially and the lateral border of brain laterally at supraventricular and high ventricular level. At the low ventricular level frontal lobes are bounded anteriorly by frontal pole, posteriorly by genu of corpus callosum and frontal horn of lateral ventricle and lateral sulcus posterolaterally. At the infraventricular level frontal lobes consist of gyrus rectus medially and orbital gyri laterally (Figure 2.4).

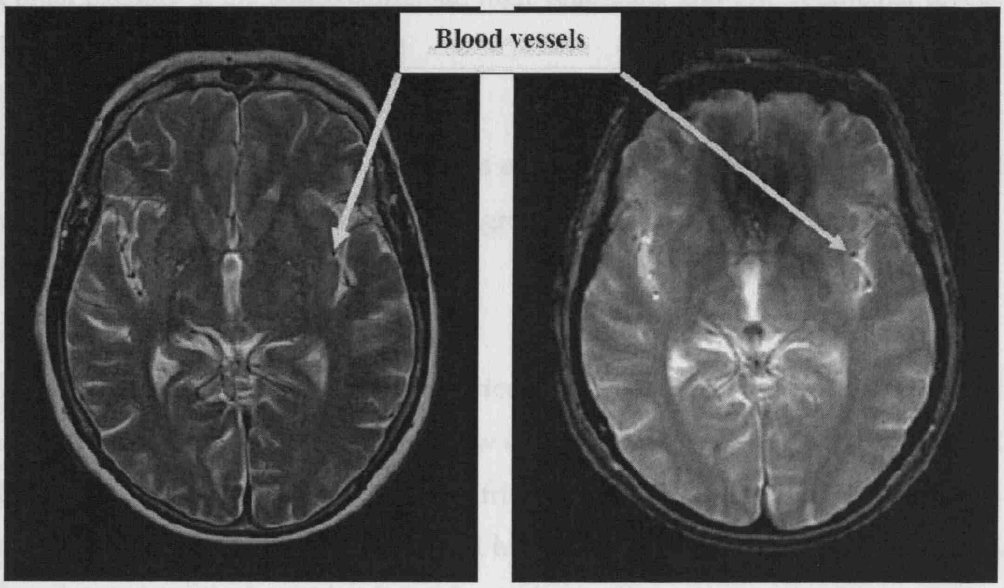
Figure 2.2: Mimics of Brain Microbleeds
Courtesy: Dr. Simone Gregoire



(i)
(A) Symmetrical hypointensities in basal ganglia (arrow)
(B) Calcifications seen on CT scan in the same places (arrow)



(ii) Frontal air bone interfaces on T2* W GRE MRI



(iii) Blood Vessels in subarachnoid space on T2W FSE and T2*W GRE MRI

The Parietal Lobes lie between cental sulcus anteriorly, parieto-occipital sulcus posteriorly, interhemispheric fissure medially and the lateral border of brain laterally at the supraventricular and high ventricular level. (Figure 2.4)

The Temporal Lobes are identified at the low ventricular and infraventricular level. The temporal lobes are bounded anteriorly by the temporal pole. Medially they are separated from the insula by the lateral sulcus at low ventricular level and at infraventricular level they are separated from the frontal lobe by sylvian cistern anteromedially. The temporal lobes are separated from the occipital lobe posteriorly by an imaginary line drawn vertically from parieto-occipital sulcus to the inferior border. The brainstem and cerebellum lies medially and posteromedially respectively to the temporal lobes. (Figure 2.4)

The Occipital Lobes lie between the parieto-occipital sulcus anteriorly, the occipital pole posteriorly, the interhemispheric fissure medially and the lateral border of brain laterally at the supraventricular, high ventricular and low ventricular levels. (Figure 2.4)

The Centrum Semiovale is defined as a central core of white matter surrounded by peripheral array of convolutions of grey matter at the supraventricular level. (Figure 2.4)

The Caudate nucleus lies at high ventricular level and low ventricular level. The tail of caudate nucleus appears as a narrow strip of grey matter along the lateral wall of the body of lateral ventricle at high ventricular level. Body of caudate nucleus lays at low ventricular level behind the frontal horns of the lateral ventricle and the head of caudate indents the lateral border of frontal horn of lateral ventricle. (Figure 2.4)

The Lentiform nucleus is a wedge shaped large mass of grey matter lying lateral to the caudate nucleus and the thalamus at the low ventricular level. It is bounded medially by the internal capsule and laterally by the external capsule. (Figure 2.4)

The Internal Capsule is defined as the L-shaped strip of white matter on the medial side of the lentiform nucleus at the low ventricular level. It has an anterior short limb which separates the caudate nucleus from the lentiform nucleus and a long posterior limb which separates the thalamus from the lentiform nucleus. (Figure 2.4)

The External Capsule is defined as the narrow strip of white matter at the low ventricular level, bounded medially by the lentiform nucleus and laterally by the insula. (Figure 2.4)

The Thalamus lies between the posterior limb of internal capsule anterolaterally, temporal lobe posterolaterally, third ventricle anteromedially and splenium of the corpus callosum posteriorly and posteromedially. (Figure 2.4)

The Insula is a part of cerebral cortex identified at the low ventricular level which is surrounded and buried by the overgrowth of the temporal, parietal and frontal lobes. Laterally it is separated from the temporal lobe by the lateral sulcus and medially it is bounded by the extreme capsule and the claustum. (Figure 2.4)

The Corpus Callosum is identified at the high and low ventricular levels. The corpus callosum interrupts the middle third of the central sulcus at the high ventricular level and is bounded by the body of lateral ventricles on each side. At the low ventricular level genu of the corpus callosum lies between the two frontal horns of lateral ventricles, bounded anteriorly by the cingulate gyrus and inter hemispheric fissure. The splenium of the corpus callosum also lies at the low ventricular level between the thalamus and the third ventricle anteriorly and the isthmus of cingulate gyrus and central sulcus posteriorly. On each side the splenium of the corpus callosum is bounded by temporal horns of lateral ventricles. (Figure 2.4)

The Midbrain, pons and medulla were classified together in the category of ***brainstem***. The midbrain lies at low ventricular level, when the section is low. The midbrain is identified by the "Micky Mouse ears" appearance of the cerebral

peduncles anterolaterally and the cerebral aqueduct posteriorly at the infraventricular level. Pons lie below the midbrain and is identified by the enlargement of cerebral aqueduct into fourth ventricle. It is bounded by the superior and middle cerebellar peduncles posterolaterally. Medulla lies below the pons. It is identified by the mushroom cap appearance because of the undersurface of the belly of the pons at higher sections and by the ventral sulcus and the most inferior portion of fourth ventricle at lower sections. (Figure 2.4)

The Cerebellum lies below the infraventricular level, in the posterior fossa, behind the brainstem. (Stark & Bradley 1999).

2.6: Statistical Analysis

All the data was entered into Statistical Package for the Social Science (SPSS) version 14. Variables including age, sex, MRI diagnosis, TE value, presence and number of total, definite and possible microbleeds in any location of the brain, in lobar, deep and infratentorial distribution and presence of definite microbleeds in individual anatomical regions by rater 1 (single reading) and rater 2 (reading 1 and reading 2) were made. Frequencies were calculated to evaluate the demographic characteristics of the data. Intra and Inter-rater agreement for the presence of microbleeds was evaluated using kappa statistics in SPSS. The kappa test was chosen because it is a good test for assessing the agreement between two ratings and can estimate whether agreement exceeds chance levels for both binary and nominal ratings. SPSS cannot calculate kappa statistics when there is an unequal range of scores in two ratings. Therefore STATA Intercooled version 8 was used to calculate kappa values (agreement) for the number of microbleeds between two ratings. Kappa statistics results were interpreted as: 0–0.20 = poor agreement, 0.21–0.40 = fair agreement, 0.41–0.60 = moderate agreement, 0.61–0.80 = good agreement and 0.81–1 = very good agreement (Landis & Koch 1977).

Figure 2.3: Brain Microbleed Rating Scale

Microbleed patient number	RS					Patient hospital number:

Inclusion criteria:	small , rounded, circular, dot like, well defined, hypointense lesions; size: 2-10 mm Two hypointensities at the same place in two successive slices of T2* will be considered as a single microbleed
Exclusion criteria:	- Any linear lesion: deep or superficial - Structures in subarachnoid spaces - Symmetrical hypointensities in the globi pallidi (likely to be calcification or iron deposition) - Flow voids from cortical vessels - Haemorrhage within an area of infarction - Small haemorrhages in close proximity to / adjacent to a primary ICH NB If only 1 microbleed present, do not discount

Rater SG ☐ UJC ☐ DW ☐ RJ ☐	Rating scale version: 1.5 (revised 280208)

Definite BMBs (L)		Possible BMBs (L)	
Definite BMBs (R)		Possible BMBs (R)	
Definite BMBs: L+R		Definite + Possible: total	

LEFT									
Lobar									
	Frontal		Temporal		Parietal		Occipital		N
	Cortical	Sub/juxta-cortical	Cortical	Sub/juxta-cortical	Cortical	Sub/juxta-cortical	Cortical	Sub/juxta- cortical	
Definite									
Possible									
Deep									
	Thalamus	Lentiform	Caudate	IC	EC	Insula	CC	CSO	N
Definite									
Possible									
Infratentorial									
	Brainstem				Cerebellum				N
Definite									
Possible									

RIGHT									
Lobar									
	Frontal		Temporal		Parietal		Occipital		N
	Cortical	Sub/juxta-cortical	Cortical	Sub/juxta-cortical	Cortical	Sub/juxta-cortical	Cortical	Sub/juxta- cortical	
Definite									
Possible									
Deep									
	Thalamus	Lentiform	Caudate	IC	EC	Insula	CC	CSO	N
Definite									
Possible									
Infratentorial									
	Brainstem				Cerebellum				N
Definite									
Possible									

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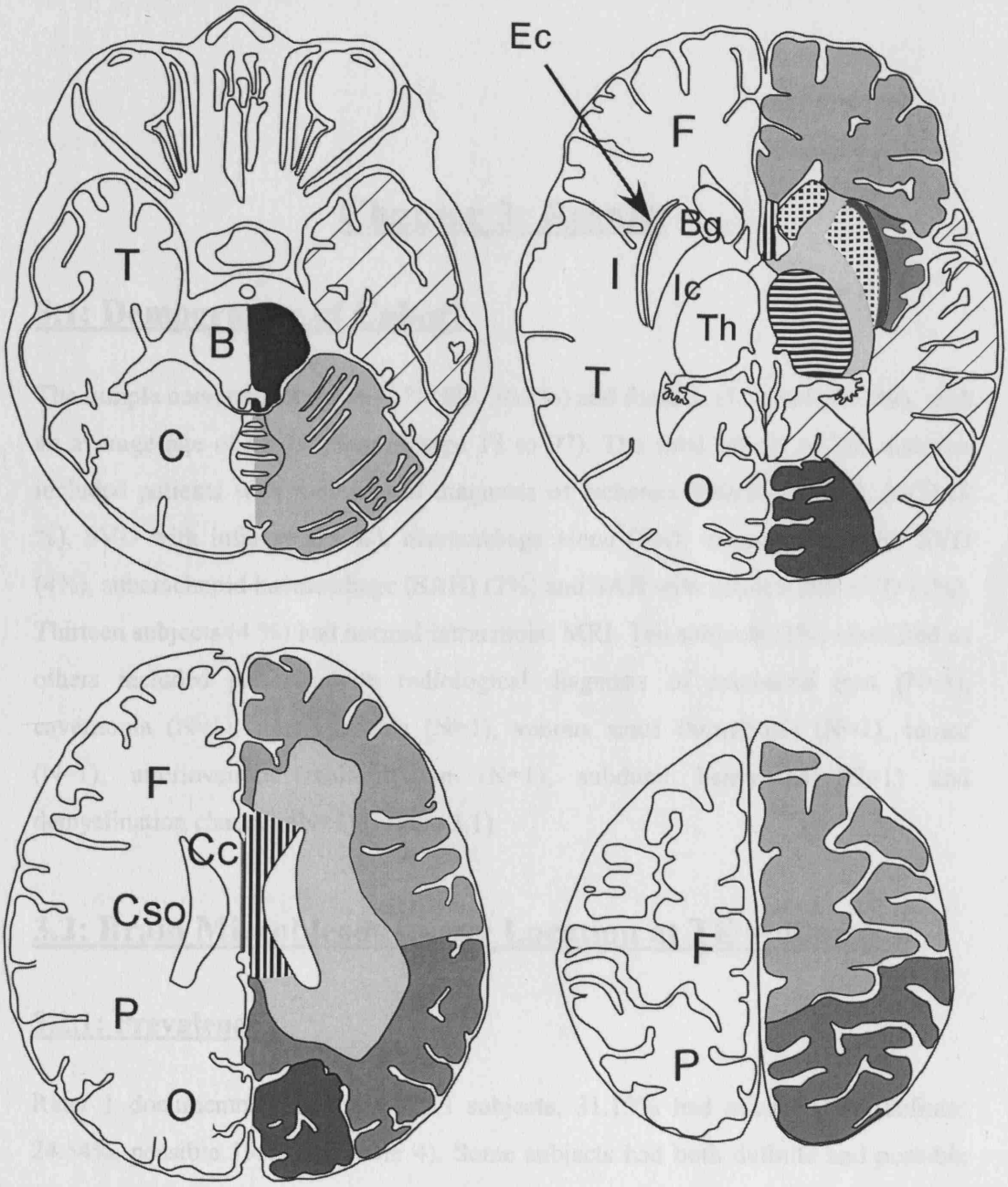


Figure 2.4: Anatomical Boundaries
Courtesy: Dr. Simone Gregoire

F = Frontal lobe P = Parietal Lobe T = Temporal Lobe
O = Occipital Lobe T = Thalamus Bg = Basal Ganglia
Cc = Corpus Callosum Cso = Centrum Semi Ovale
I = Insula Ic = Internal Capsule Ec = External Capsule
B = Brainstem C = Cerebellum

Chapter 3: Results

3.1: Demography of Cohort

The sample consisted of males (172/303, 56.8%) and females (131/303, 43.2%), with an average age of 65.33 years (Range 18 to 97). The final cohort of 303 subjects included patients with radiological diagnosis of Ischemic Infarcts (37 %), SVD (9 %), SVD with infarcts (35 %), haemorrhage alone (5%), haemorrhage with SVD (4%), subarachnoid haemorrhage (SAH) (2%) and SAH with infarcts and SVD (1%). Thirteen subjects (4 %) had normal intracranial MRI. Ten subjects (3%) classified as others included patients with radiological diagnosis of arachnoid cyst (N=3), cavernoma (N=1), meningocoele (N=1), venous sinus thrombosis (N=1), tumor (N=1), arteriovenous malformation (N=1), subdural hematoma (N=1) and demyelination changes (N=1) (Figure 3.1).

3.2: Brain Microbleeds in any Location at TE = 40msec

3.2.1: Prevalence

Rater 1 documented that out of 273 subjects, 31.13% had microbleeds (definite 24.54%, possible 19.41%) (Table 4). Some subjects had both definite and possible microbleeds. The number of total microbleeds counted by rater 1 in any location of the brain was 379 including both definite (N=267) and possible (N=112) microbleeds (Figure 3.2).

In the first rating, rater 2 found that 36.63% subjects had microbleeds (definite 30.76%, possible 18.68%) (Table 4). Rater 2 counted 458 microbleeds in total, including both definite (N=346) and possible (N=112) microbleeds (Figure 3.2).

In the second rating by Rater 2, 38.46% subjects were noticed to have microbleeds (definite 31.13%, possible 23.44%) (Table 4). The total number of microbleeds counted by Rater 2 was 510, including both definite (N=358) and possible (N=152) ones (Figure 3.2).

3.2.2: Intra and Inter-rater Agreement

The intra-rater agreement for the presence of definite, possible and total microbleeds in any location of the brain was, κ = 0.87 (95% CI, 0.75-0.99), κ = 0.72 (95% CI, 0.60-0.84) and κ = 0.84 (95% CI, 0.72-0.96), respectively (Table 5). The inter-rater agreement for the presence of definite, possible and total microbleeds in any location of the brain was, κ = 0.69 (95% CI, 0.57-0.81), κ = 0.31 (95% CI, 0.19-0.43) and κ = 0.61 (95% CI, 0.49-0.73), respectively (Table 5).

Rater 1 and 2 did not agree for the presence of microbleeds in 48/273 subjects. The MRI scans of these subjects were reviewed with the arbitrators (DW and RJ). It was observed that disagreement between rater 1 and 2 occurred for majority of those subjects where there was only one microbleed (42/48).

The intra and inter-rater agreement for the number of definite microbleeds in any location of the brain was found to be moderate to good, and was fair to moderate for the number of possible and total microbleeds (Appendix 1).

Figure 3.1: Sample Distribution based on Radiological Diagnosis

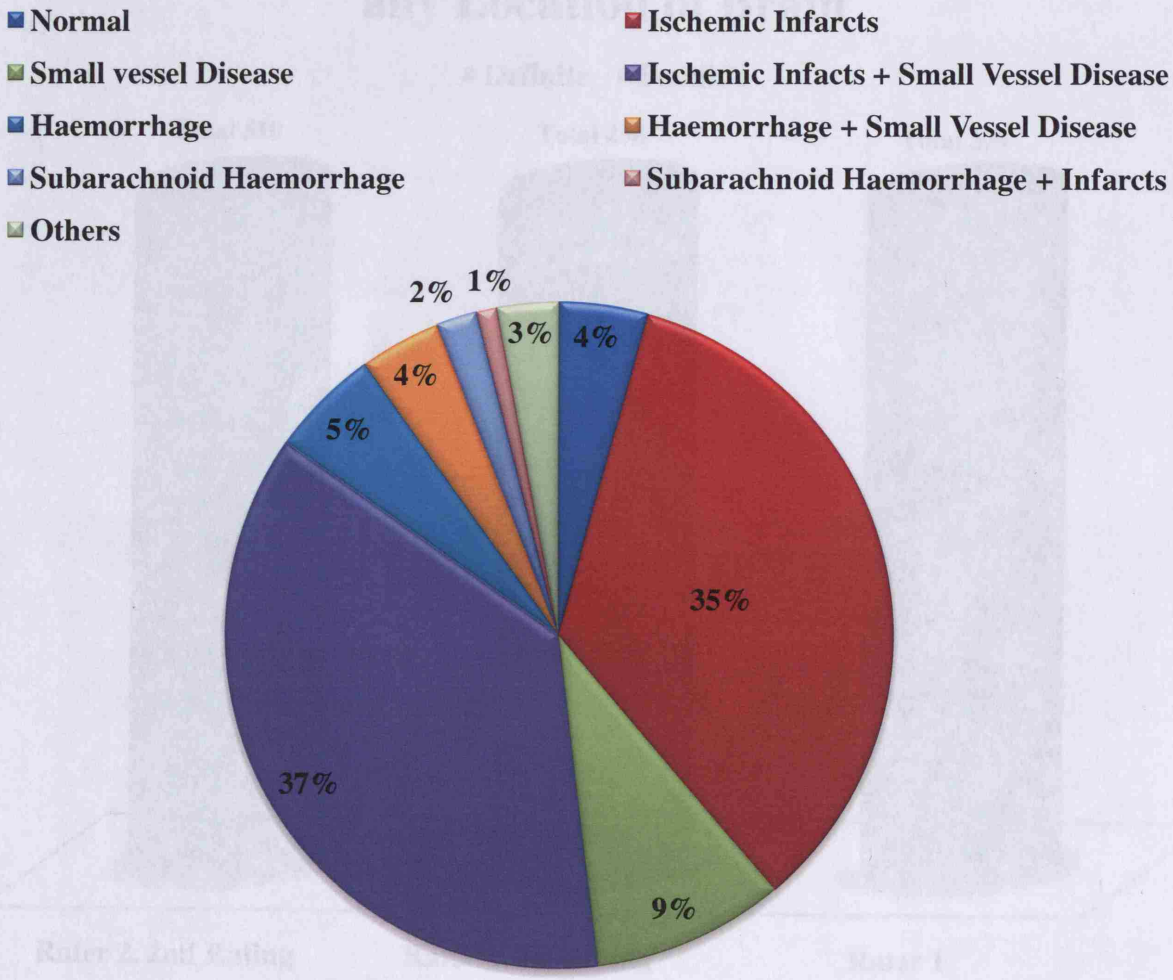


Figure 3.2: Number of Brain Microbleeds in any Location of Brain

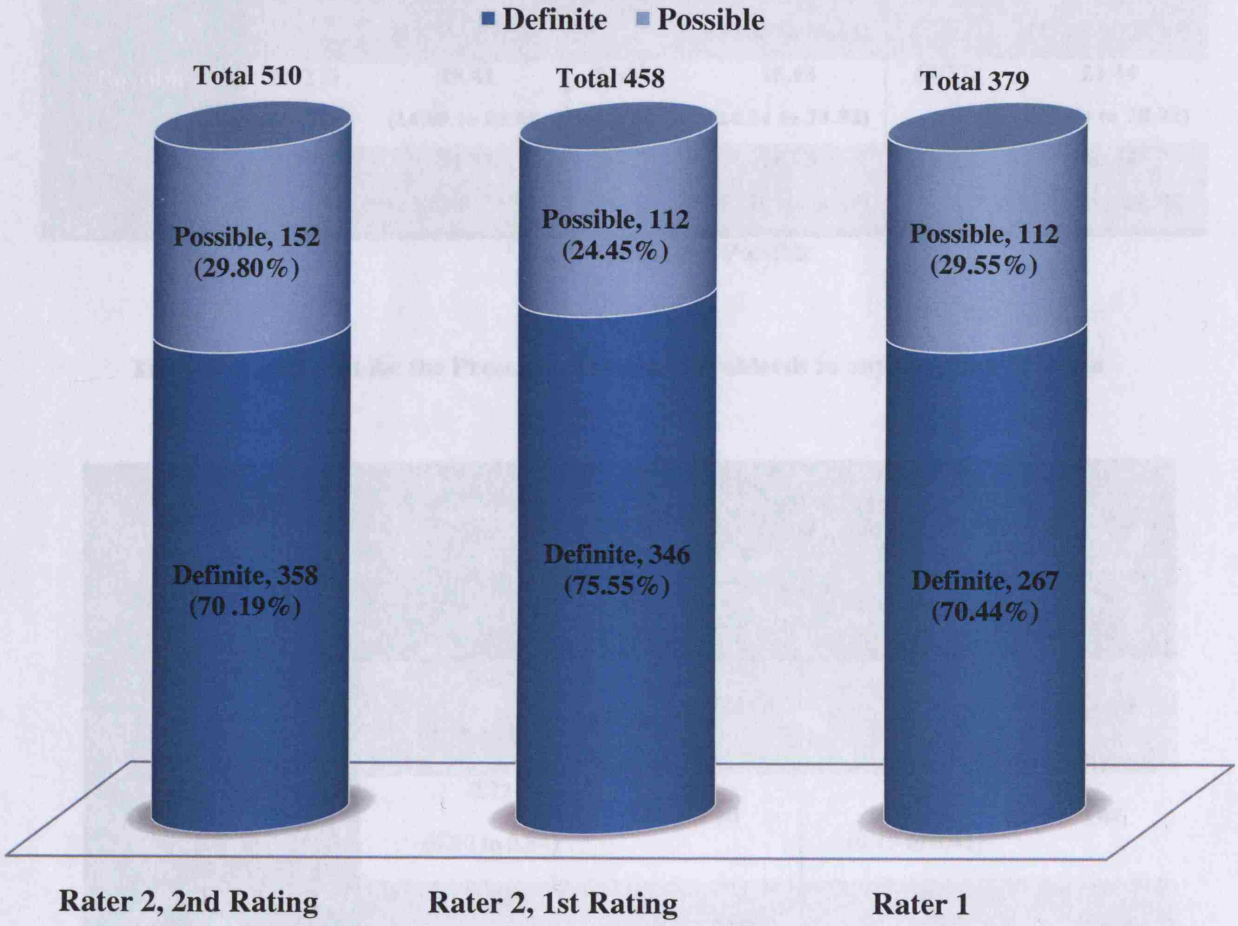


Table 4: Prevalence of Brain Microbleeds in any Location

Brain Microbleeds	Prevalence					
	Rater 1		Rater 2, 1st Rating		Rater 2, 2nd Rating	
	No. of Subjects	% (95% CI)	No. of Subjects	% (95% CI)	No. of Subjects	% (95% CI)
Definite	67/273	24.54 (19.55 to 30.1)	84/273	30.76 (25.35 to 36.61)	85/273	31.13 (25.69 to 36.99)
Possible	53/273	19.41 (14.89 to 24.61)	51/273	18.68 (14.24 to 23.82)	64/273	23.44 (18.54 to 28.92)
Total*	85/273	31.13 (25.69 to 36.99)	100/273	36.63 (30.90 to 42.65)	105/273	38.46 (32.66o 44.51)

*Total = Definite + Possible

Table 5: Agreement for the Presence of Brain Microbleeds in any Location of Brain

Presence of Brain Microbleeds	Intra-rater Agreement K (95% CI)	Level of Agreement	Inter-rater Agreement K (95% CI)	Level of Agreement
Definite	0.87 (0.75 to 0.99)	Very Good	0.69 (0.57 to 0.81)	Good
Possible	0.72 (0.60 to 0.84)	Good	0.32 (0.19 to 0.43)	Fair
Total*	0.84 (0.72 to 0.96)	Very Good	0.61 (0.49 to 0.73)	Good

*Total = Definite + Possible

3.3: Brain Microbleeds in Lobar, Deep and Infratentorial Distribution at TE = 40msec

3.3.1: Prevalence

Rater 1 found that, out of 273 subjects, 21.2% had lobar (definite + possible), 19.4% had deep (definite + possible) and 8.8% had infratentorial (definite + possible) microbleeds (table 6). Rater 1 documented 217 microbleeds (definite = 152, possible = 65) in the lobar, 123 (definite = 86, possible = 37) in the deep and 39 (definite = 29, possible = 10) in the infratentorial regions (Figure 3.3).

In the first rating, Rater 2 noticed that, out of 273 subjects, 27.4% had lobar (definite + possible), 18.3% had deep (definite + possible) and 13.9% had infratentorial microbleeds (definite + possible) (table 6). Rater 2 counted 267 microbleeds (definite = 190, possible = 77) in the lobar, 126 (definite = 106, possible = 20) in the deep and 65 (definite = 50, possible = 15) in the infratentorial regions (Figure 3.3).

For the second rating, Rater 2 documented that out of 273 subjects, 26% had lobar (definite + possible), 21.1% had deep (definite + possible) and 16.8% had infratentorial microbleeds (definite + possible) (table 6). Rater 2 counted 293 lobar (definite = 201, possible = 92), 140 deep (definite = 109, possible = 31) and 77 infratentorial microbleeds (definite = 48, possible = 29) (Figure 3.3).

In summary both raters documented that the number of microbleeds was highest in the lobar distribution followed by the deep and the infratentorial regions (Figure 3.3).

3.3.2: Intra and Inter-rater Agreement

The intra-rater agreement for the presence of definite microbleeds in the lobar, deep and infratentorial distribution was K = 0.83 (95% CI, 0.71-0.95), K = 0.94 (95% CI, 0.82-1) and K = 0.90 (95% CI, 0.78-1), respectively (table7). The inter-rater agreement for the presence of definite microbleeds in the lobar, deep and infratentorial distribution was K = 0.74 (95% CI, 0.62-0.86), K = 0.67 (95% CI, 0.55-0.79) and K = 0.74 (95% CI, 0.62-0.86), respectively (table7). The intra and inter-rater agreement for the presence of possible microbleeds in the same regions ranged from fair to good, and was good to very good for the presence of total (definite + possible) microbleeds (see Appendix1).

The intra and inter-rater agreement for the number of definite and total (definite + possible) microbleeds in the lobar, deep and infratentorial regions was moderate to very good, and poor to moderate for the number of possible microbleeds in the same locations (Appendix1).

Table 6: Prevalence of Brain Microbleeds in Lobar, Deep and Infratentorial Distribution

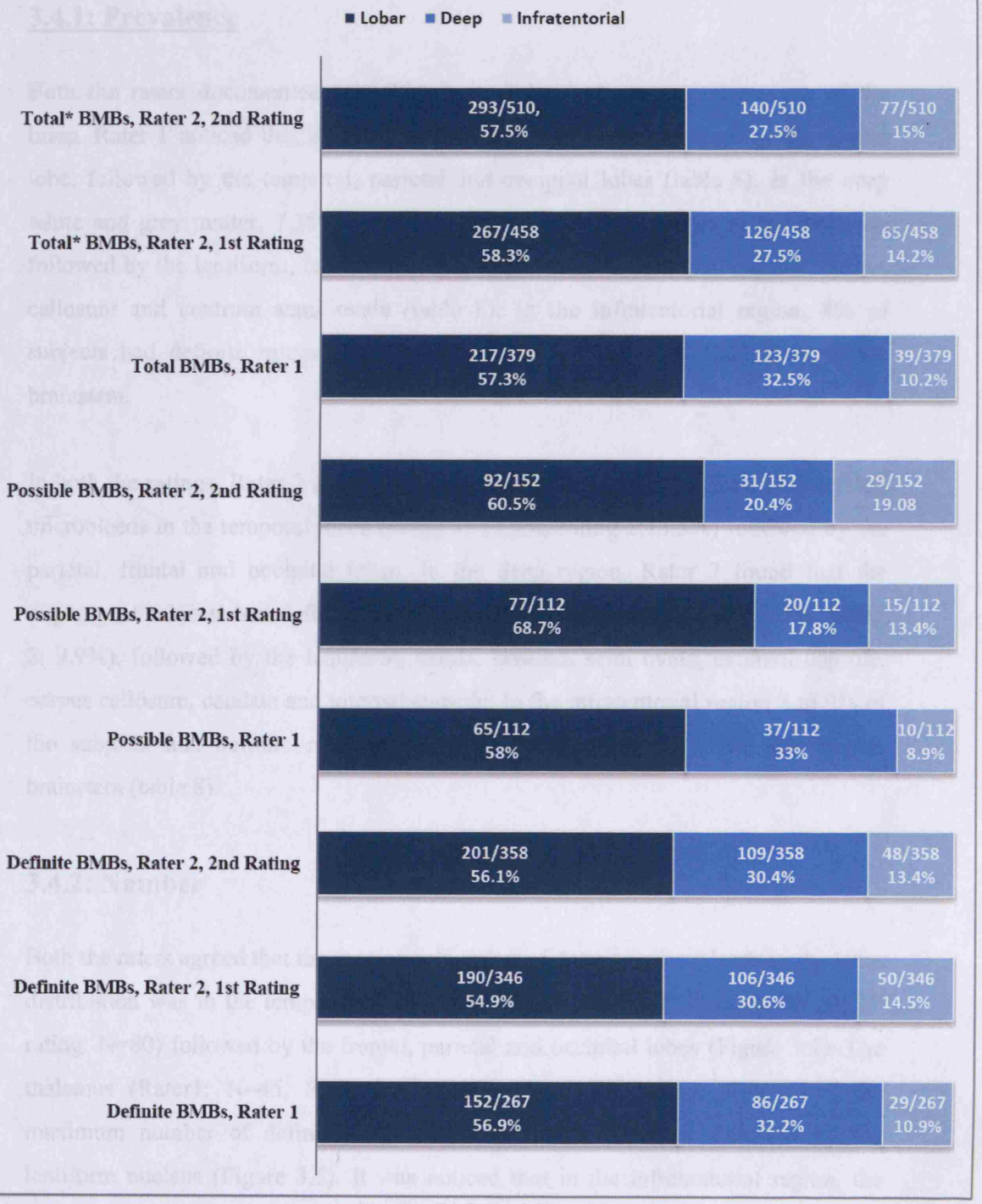
Location	Prevalence					
	Rater 1		Rater 2, 1st Rating		Rater 2, 2nd Rating	
	No. of Subjects	% (95% CI)	No. of Subjects	% (95% CI)	No. of Subjects	% (95% CI)
Definite Lobar	44/273	16.1% (11.96 to 21.03)	55/273	21.1% (15.55 to 25.4)	58/273	21.2% (16.5 to 26.6)
Definite Deep	40/273	14.6% (10.68 to 19.41)	43/273	15.8% (11.64 to 20.62)	43/273	15.8% (11.6 to 20.6)
Definite Infratentorial	18/273	6.6% (3.95 to 10.22)	28/273	10.2% (6.92 to 14.48)	29/273	10.6% (7.2 to 14.9)
Possible Lobar	32/273	11.7% (8.16 to16.14)	38/273	13.9% (10.04 to 18.6)	39/273	14.2% (10.3 to 19)
Possible Deep	21/273	7.7% (4.82 to 11.52)	16/273	5.8% (3.39 to 9.34)	23/273	8.4% (5.4 to 12.3)
Possible Infratentorial	9/273	3.3% (1.52 to 6.16)	13/273	4.7% (2.56 to 8)	22/273	8% (5.1 to 11.9)
Total* Lobar	58/273	21.2% (16.54 to 26.58)	75/273	27.4% (22.26 to 33.17)	71/273	26% (20.9 to 31.6)
Total * Deep	53/273	19.4% (14.89 to 24.61)	50/273	18.3% (13.9 to 23.42)	55/273	21.1% (15.5 to 25.4)
Total* Infratentorial	24/273	8.8% (5.71 to 12.8)	38/273	13.9% (10.04 to 18.6)	46/273	16.8% (12.6 to 21.8)

*Total = Definite + Possible

Table 7: Agreement for the Presence of Definite Brain Microbleeds in Lobar, Deep and Infratentorial Distribution

Definite Brain Microbleeds	Intra-rater Agreement Ƙ (95% CI)	Level of Agreement	Inter-rater Agreement Ƙ (95% CI)	Level of Agreement
Lobar	0.83 (0.71 to 0.95)	Very Good	0.74 (0.62 to 0.86)	Good
Deep	0.94 (0.82 to 1)	Very Good	0.67 (0.55 to 0.79)	Good
Infratentorial	0.90 (0.78 to 1)	Very Good	0.74 (0.62 to 0.86)	Good

Figure 3.3: Number of Brain Microbleeds in Lobar, Deep and Infratentorial Distribution



*Total = Definite + Possible

3.4: Definite Microbleeds in Individual Anatomical Regions of Brain at TE = 40 msec

3.4.1: Prevalence

Both the raters documented microbleeds in individual anatomical regions of the brain. Rater 1 noticed that 8.9% of subjects had definite microbleeds in the frontal lobe, followed by the temporal, parietal and occipital lobes (table 8). In the deep white and grey matter, 7.3% of subjects had definite microbleeds in the thalamus followed by the lentiform, caudate, external capsule, insula, internal capsule, corpus callosum and centrum semi ovale (table 8). In the infratentorial region, 4% of subjects had definite microbleeds in the cerebellum and 3.3% had them in the brainstem.

In both the ratings, Rater 2 noticed that the highest number of subjects had definite microbleeds in the temporal lobes (rating 1: 11.7%, rating 2:13.5%) followed by the parietal, frontal and occipital lobes. In the deep region, Rater 2 found that the majority of subjects had definite microbleeds in the thalamus (rating 1: 9.5%, rating 2: 9.9%), followed by the lentiform, insula, centrum semi ovale, external capsule, corpus callosum, caudate and internal capsule. In the infratentorial region 8 to 9% of the subjects had definite microbleeds in the cerebellum and 3.6% had in the brainstem (table 8).

3.4.2: Number

Both the raters agreed that the maximum number of definite microbleeds in the lobar distribution was in the temporal lobes (Rater1: N=51, Rater 2:1st rating: N=70, 2nd rating: N=80) followed by the frontal, parietal and occipital lobes (Figure 3.4). The thalamus (Rater1: N=45, Rater 2:1st rating: N=56, 2nd rating: N=57) had the maximum number of definite microbleeds in the deep region followed by the lentiform nucleus (Figure 3.5). It was noticed that in the infratentorial region, the cerebellum had the maximum number of definite microbleeds (Rater1: N=19, Rater

2:1st rating: N=38, 2nd rating: N=35) followed by the brainstem (Rater1: N=10, Rater 2:1st rating: N=13, 2nd rating: N=13) (Figure 3.6).

3.4.3: Intra and Inter-rater Agreement

The intra-rater agreement for the presence of definite microbleeds in the frontal, temporal, parietal and occipital lobe was good to very good [κ (95% CI) = 0.89 (0.77-1), 0.85 (0.73-1), 0.98 (0.86-1), 0.67 (0.55-79), respectively]. The inter-rater agreement was good for the frontal, temporal and parietal lobes [κ (95% CI) = 0.77 (0.65-0.89), 0.72 (0.60-0.84), 0.74 (0.62-0.86), respectively] and moderate for the occipital lobe [κ (95% CI) = 0.60 (0.48-0.72)] (Appendix 3).

The intra-rater agreement for the presence of definite microbleeds in the thalamus, lentiform, brainstem and cerebellum was very good [κ (95% CI) = 0.94 (0.82-1), 0.84 (0.72-0.96), 1 (0.88-1), 0.84 (0.72-0.96), respectively] (Appendix 3). The inter-rater agreement for the presence of definite microbleeds was very good for the thalamus and the brainstem [κ (95% CI) = 0.84 (0.72-0.96), 0.94 (0.82-1), respectively] and moderate for the lentiform and the cerebellum [κ (95% CI) = 0.42 (0.30-0.54), 0.59 (0.47-0.71), respectively] (Appendix 3).

There were only a few cases with microbleeds in insula, centrum semi ovale, external capsule, corpus callosum, caudate and internal capsule, therefore, intra and inter-rater agreement were not calculated for these locations.

Table 8: Prevalence of Definite Brain Microbleeds in Individual Anatomical Regions

Location	Prevalence					
	Rater 1		Rater 2, 1st Rating		Rater 2 2nd Rating	
	No. of Subjects	% 95% CI	No. of Subjects	% 95% CI	No. of Subjects	% 95% CI
Frontal Lobe	24/273	8.9 5.7 to 12.8	25/273	9.1 6 to 13.2	24/273	8.9 5.7 to 12.8
Temporal Lobe	22/273	8 5.1 to 11.9	32/273	11.7 8.2 to 16.1	37/273	13.5 9.7 to 18.1
Parietal Lobe	21/273	7.7 4.8 to 11.5	27/273	9.8 6.6 to 14.1	26/273	9.5 6.3 to 13.6
Occipital Lobe	13/273	4.8 2.6 to 8	13/273	4.8 2.6 to 8	16/273	5.9 3.3 to 9.3
Thalamus	20/273	7.3 4.5 to 11.1	26/273	9.5 6.3 to 13.6	27/273	9.9 6.6 to 14.1
Lentiform	18/273	6.6 3.9 to 10.2	16 /273	5.9 3.3 to 9.3	17/273	6.2 3.7 to 9.7
Caudate	3/273	1.1 0.2 to 3.2	2/273	0.7 0.09 to 2.6	2/273	0.7 0.09 to 2.6
Internal Capsule	2/273	0.7 0.09 to 2.6	1/273	0.4 0.009 to 2	1/273	0.4 0.009 to 2
External Capsule	3/273	1.1 0.2 to 3.2	3/273	1.1 0.2 to 3.2	3/273	1.1 0.2 to 3.2
Insula	3/273	1.1 0.2 to 3.2	7/273	2.6 1 to 5.2	8/273	2.9 1.3 to 5.7
Corpus Callosum	2/273	0.7 0.09 to 2.6	2/273	0.7 0.09 to 2.6	2/273	0.7 0.09 to 2.6
Centrum Semi Ovale	2/273	0.7 0.09 to 2.6	4/273	1.4 0.4 to 3.7	5/273	1.8 0.6 to 4.2
Brainstem	9/273	3.3 1.5 to 6.2	10/273	3.6 1.8 to 6.6	10/273	3.6 1.8 to 6.6
Cerebellum	11/273	4 2 to 7	24/273	8.87 5.7 to 12.8	25/273	9.1 6 to 13.2

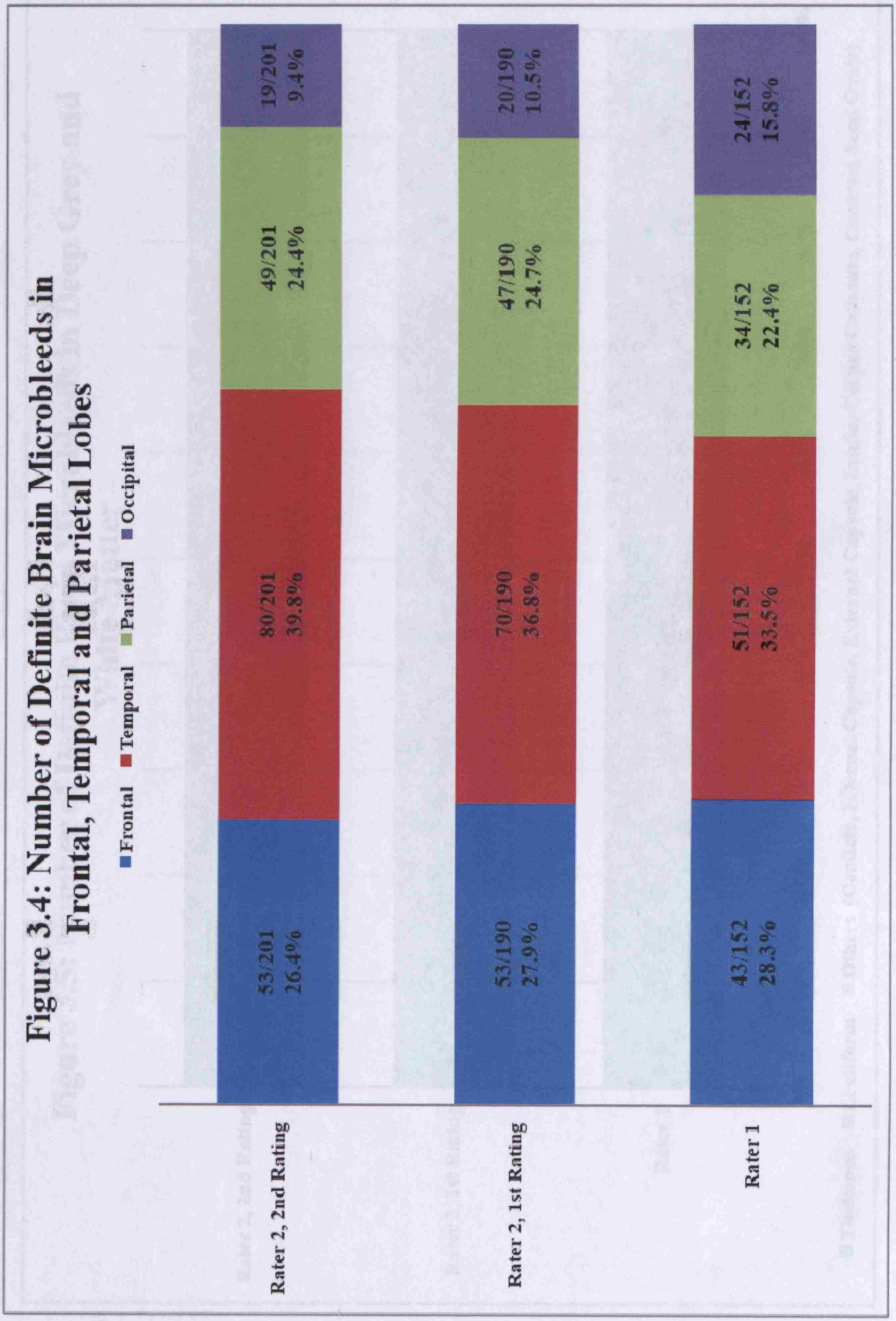


Figure 3.4: Number of Definite Brain Microbleeds in Frontal, Temporal and Parietal Lobes
Frontal
Temporal
Parietal
Occipital
Rater 2, 2nd Rating
53/201
26.4%
80/201
39.8%
49/201
24.4%
19/201
9.4%
Rater 2, 1st Rating
53/190
27.9%
70/190
36.8%
47/190
24.7%
20/190
10.5%
Rater 1
43/152
28.3%
51/152
33.5%
34/152
22.4%
24/152
15.8%

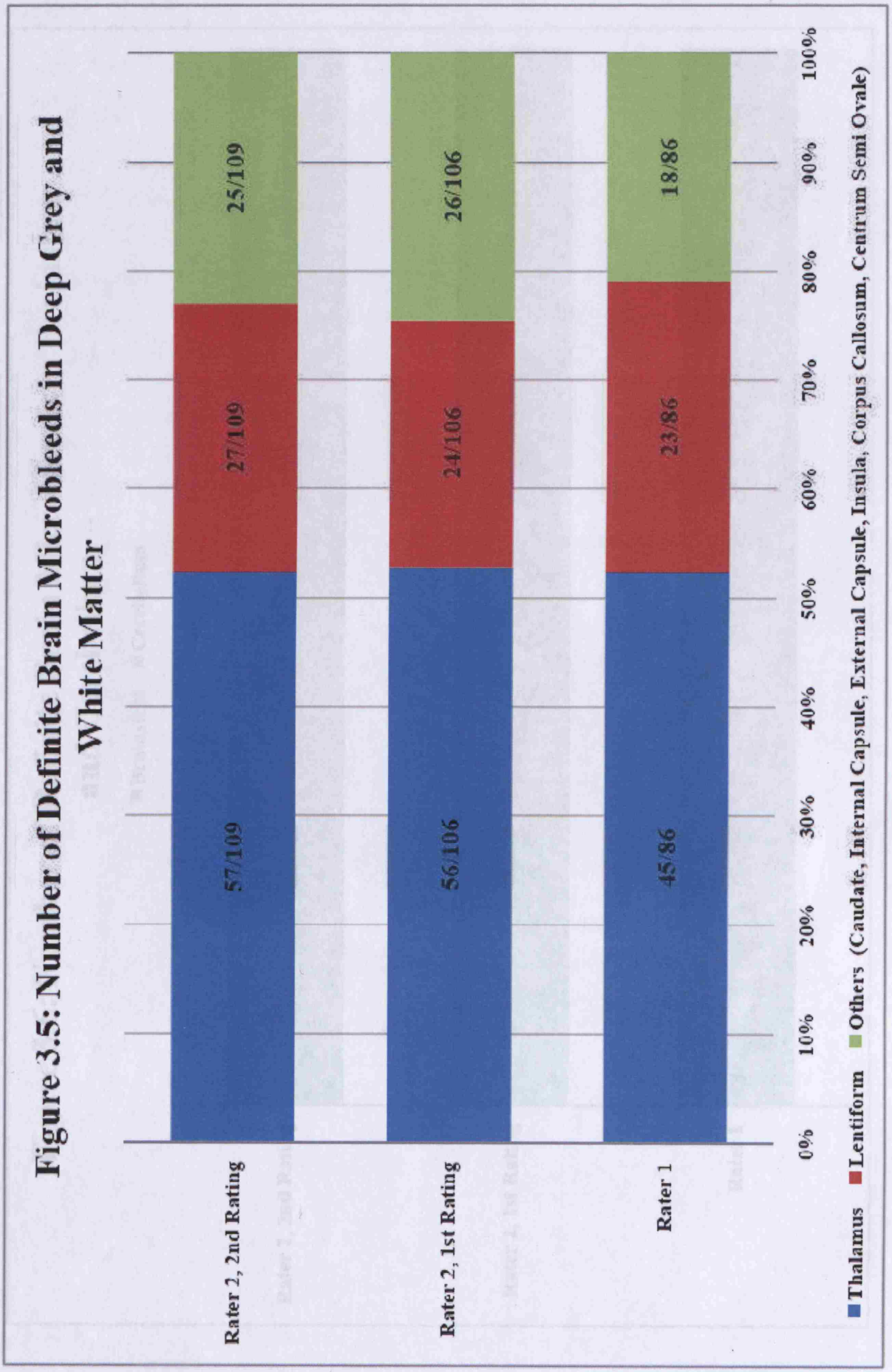


Figure 3.5: Number of Definite Brain Microbleeds in Deep Grey and White Matter
Rater 2, 2nd Rating
Rater 2, 1st Rating
Rater 1
57/109
27/109
25/109
56/106
24/106
26/106
45/86
23/86
18/86
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
Thalamus
Lentiform
Others (Caudate, Internal Capsule, Insula, Corpus Callosum, Centrum Semi Ovale)

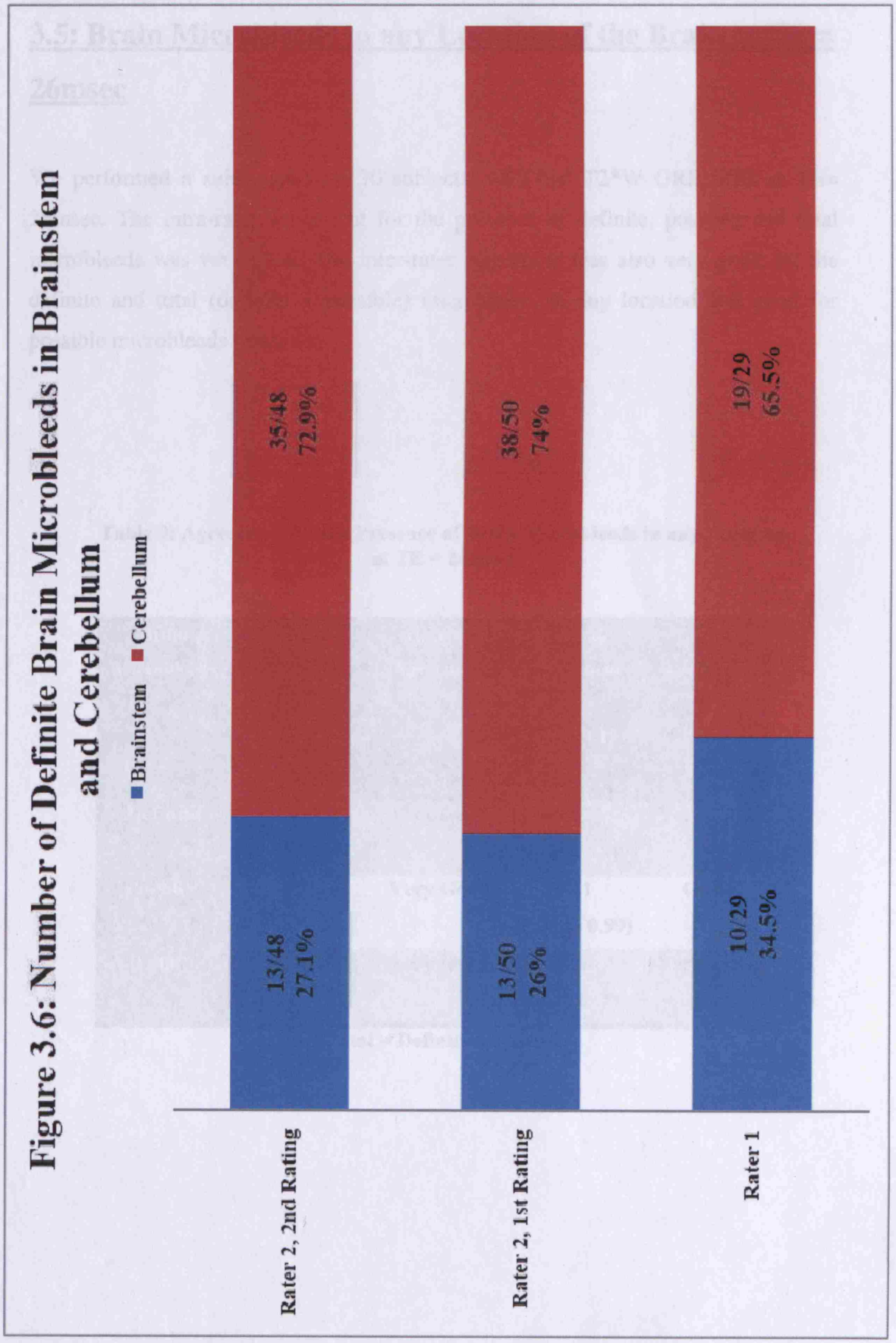


Figure 3.6: Number of Definite Brain Microbleeds in Brainstem and Cerebellum
Brainstem
Cerebellum
13/48
27.1%
35/48
72.9%
13/50
26%
38/50
74%
10/29
34.5%
19/29
65.5%
Rater 2, 2nd Rating
Rater 2, 1st Rating
Rater 1

3.5: Brain Microbleeds in any Location of the Brain at TE = 26msec

We performed a subanalysis on 30 subjects, who had T2*W GRE MRI at TE= 26msec. The intra-rater agreement for the presence of definite, possible and total microbleeds was very good. The inter-rater agreement was also very good for the definite and total (definite + possible) microbleeds in any location and good for possible microbleeds (table 9).

Table 9: Agreement for the Presence of Brain Microbleeds in any Location at TE = 26msec

Brain Microbleeds	Intra-rater Agreement κ (95% CI)	Level of Agreement	Inter-rater Agreement κ (95% CI)	Level of Agreement
Definite	1	Very Good	0.87 (0.61 to 1)	Very Good
Possible	1	Very Good	0.61 (0.23 to 0.99)	Good
Total*	1	Very Good	0.88 (0.66 to 1)	Very Good

*Total = Definite + Possible

Appendix 1: Intra and Inter-rater Agreement for the Presence and Number of Brain Microbleeds in any Location of the Brain

	Presence of Brain Microbleeds						Number of Brain Microbleeds					
Brain Microbleeds	Intra-rater Agreement		Inter-rater Agreement				Intra-rater Agreement		Inter-rater Agreement			
	Rater 2 1st Rating & 2nd Rating K (95%CI)	Level of Agreement	Rater 1 & Rater 2 (1st Rating) K (95%CI)	Level of Agreement	Rater 1 & Rater 2 (2nd Rating) K (95%CI)	Level of Agreement	Rater2 1st Rating & 2nd Rating K (95%CI)	Level of Agreement	Rater 1 & Rater 2 (1st Rating) K (95%CI)	Level of Agreement	Rater 1 & Rater 2 (2nd Rating) K (95%CI)	Level of Agreement
Definite	**0.87 (0.75 to 0.99)**	**Very Good**	**0.68 (0.56 to 0.80)**	**Good**	**0.69 (0.57 to 0.81)**	**Good**	**0.71 (0.65 to 0.77)**	**Good**	**0.44 (0.37 to 0.51)**	**Moderate**	**0.48 (0.41 to 0.55)**	**Moderate**
Possible	0.72 (0.60 to 0.84)	Good	0.38 (0.26 to 0.50)	Fair	0.31 (0.19 to 0.43)	Fair	0.49 (0.43 to 0.55)	Moderate	0.22 (0.14 to 0.30)	Fair	0.23 (0.15 to 0.31)	Fair
Total*	0.84 (0.72 to 0.96)	Very Good	0.58 (0.46 to 0.70)	Moderate	0.61 (0.49 to 0.73)	Good	0.60 (0.54 to 0.66)	Moderate	0.39 (0.33 to 0.45)	Fair	0.39 (0.33 to 0.45)	Fair

***Total = Definite + Possible**

Appendix 2: Intra and Inter-rater Agreement for the Presence and Number of Brain Microbleeds in Lobar, Deep and Infratentorial Distribution

Brain Microbleeds	Presence of Brain Microbleeds						Number of Brain Microbleeds					
	Intra-rater Agreement		Inter-rater Agreement				Intra-rater Agreement		Inter-rater Agreement			
	Rater 2 1st Rating & 2nd Rating K (95%CI)	Level of Agreement	Rater 1 & Rater 2 (1st Rating) K (95%CI)	Level of Agreement	Rater 1 & Rater 2 (2nd Rating) K (95%CI)	Level of Agreement	Rater2 1st Rating & 2nd Rating K (95%CI)	Level of Agreement	Rater 1 & Rater 2 (1st Rating) K (95%CI)	Level of Agreement	Rater 1 & Rater 2 (2nd Rating) K (95%CI)	Level of Agreement
Definite Lobar	**0.83 (0.71 to 0.95)**	**Very Good**	**0.69 (0.57 to 0.81)**	**Good**	**0.74 (0.62 to 0.86)**	**Good**	**0.71 (0.64 to 0.78)**	**Good**	**0.48 (0.41 to 0.55)**	**Moderate**	**0.53 (0.46 to 0.60)**	**Moderate**
Definite Deep	**0.94 (0.82 to 1)**	**Very Good**	**0.67 (0.55 to 0.79)**	**Good**	**0.67 (0.55 to 0.79)**	**Good**	**0.84 (0.76 to 0.92)**	**Very Good**	**0.52 (0.44 to 0.60)**	**Moderate**	**0.53 (0.45 to 0.61)**	**Moderate**
Definite Infratentorial	**0.90 (0.78 to 1)**	**Very Good**	**0.76 (0.64 to 0.88)**	**Good**	**0.74 (0.62 to 0.86)**	**Good**	**0.85 (0.76 to 0.94)**	**Very Good**	**0.60 (0.51 to 0.69)**	**Moderate**	**0.61 (0.51 to 0.71)**	**Good**
Possible Lobar	0.77 (0.65 to 0.89)	Good	0.34 (0.22 to 0.46)	Fair	0.40 (0.28 to 0.52)	Fair	0.65 (0.56 to 0.74)	Good	0.23 (0.14 to 0.32)	Fair	0.29 (0.21 to 0.37)	Fair
Possible Deep	0.59 (0.47 to 0.71)	Moderate	0.22 (0.10 to 0.34)	Fair	0.21 (0.09 to 0.33)	Fair	0.48 (0.38 to 0.58)	Moderate	0.14 (0.04 to 0.24)	Poor	0.17 (0.07 to 0.27)	Poor
Possible Infratentorial	0.54 (0.42 to 0.66)	Moderate	0.15 (0.03 to 0.27)	Poor	0.22 (0.11 to 0.33)	Fair	0.46 (0.36 to 0.56)	Moderate	0.15 (0.04 to 0.26)	Poor	0.19 (0.09 to 0.29)	Poor
Total* Lobar	0.83 (0.71 to 0.95)	Very Good	0.63 (0.51 to 0.75)	Good	0.67 (0.55 to 0.79)	Good	0.65 (0.59 to 0.71)	Good	0.46 (0.39 to 0.53)	Moderate	0.51 (0.43 to 0.59)	Moderate
Total* Deep	0.87 (0.75 to 0.99)	Very Good	0.65 (0.53 to 0.77)	Good	0.68 (0.56 to 0.80)	Good	0.77 (0.69 to 0.85)	Good	0.50 (0.42 to 0.58)	Moderate	0.51 (0.43 to 0.59)	Moderate
Total* Infratentorial	0.80 (0.68 to 0.92)	Good	0.64 (0.52 to 0.76)	Good	0.58 (0.47 to 0.69)	Moderate	0.70 (0.61 to 0.79)	Good	0.52 (0.43 to 0.61)	Moderate	0.45 (0.36 to 0.54)	Moderate

***Total = Definite + Possible**

Appendix 3: Intra and Inter-rater Agreement for the Presence of Definite Brain Microbleeds in Individual Anatomical Regions

Brain Microbleeds	Intra-rater Agreement K (95%CI)	Level of Agreement	Inter-rater Agreement Rater 1 & Rater 2, 1st Rating K (95%CI)	Level of Agreement	Inter-rater Agreement Rater 1 & Rater 2, 2nd Rating K (95%CI)	Level of Agreement
Definite Frontal	0.89 (0.77 to 1)	Very Good	0.66 (0.54 to 0.78)	Good	0.77 (0.65 to 0.89)	Good
Definite Temporal	0.85 (0.73 to 0.97)	Very Good	0.75 (0.63 to 0.87)	Good	0.72 (0.60 to 0.84)	Good
Definite Parietal	0.98 (0.86 to 1)	Very Good	0.73 (0.61 to 0.85)	Good	0.74 (0.62 to 0.86)	Good
Definite Occipital	0.67 (0.55 to 0.79)	Good	0.76 (0.64 to 0.88)	Good	0.60 (0.48 to 0.72)	Moderate
Definite Thalamus	0.94 (0.82 to 1)	Very Good	0.86 (0.74 to 0.98)	Very Good	0.84 (0.72 to 0.96)	Very Good
Definite Lentiform	0.84 (0.72 to 0.96)	Very Good	0.37 (0.25 to 0.49)	Moderate	0.42 (0.30 to 0.54)	Moderate
Definite Brainstem	1	Very Good	0.94 (0.82 to 1)	Very Good	0.94 (0.82 to 1)	Very Good
Definite Cerebellum	0.84 (0.72 to 0.96)	Very Good	0.61 (0.50 to 0.72)	Good	0.59 (0.48 to 0.70)	Moderate

Chapter 4: Discussion

In the current project, intra and inter-rater agreement for the presence, number and anatomical distribution of microbleeds was systematically evaluated using a microbleed anatomical rating scale. The sample consisted of subjects admitted through an acute stroke unit. We found generally good intra and inter-rater agreement for the presence of definite microbleeds. Although assessment of intra and inter-rater agreement for the presence of microbleeds has been briefly mentioned in the methods of a few studies in the past (Fan et al. 2004;Jeerakathil et al. 2004;Kwa et al. 1998;Vernooij et al. 2008b), to the best of our knowledge there has been no previous systematic study on the development of a detailed anatomical rating scale for microbleeds with investigation of both intra and inter-rater agreement. Furthermore, we included a population representative of an acute stroke unit and tested the intra and inter-rater agreement in observers of varying range of experience.

4.1: Strengths of the Project

4.1.1: Sample Characteristics

Previously published studies have been limited by small sample size or by selected group of patients, restricting the generalizability of their findings. Kwa studied 221 patients with ischemic stroke, myocardial infarction or peripheral vascular disease (Kwa et al. 1998). Fan included 82 patients with lacunar infarcts only (Fan et al. 2004); and Jeerakathil reported on 222 patients from a healthy community based sample (Jeerakathil et al. 2004). Lee analyzed 70 unselected, consecutive patients with ICH in one study (Lee et al. 2005), and 16 patients with arterial hypertension and 10 patients with CAA in another study (Lee et al. 2007). Vernooij analyzed

normal elderly population and calculated inter-rater reliability for 500 subjects and intra-rater reliability for 300 subjects in one study (Vernooij et al. 2008b) and 200 subjects for inter-rater reliability only in a second study (Vernooij et al. 2008a). Sveinbjornsdottir studied a community based sample and calculated intra-rater reliability for 19 subjects (S S et al. 2008). Cordonnier had 264 subjects with stroke in the pilot study and 156 subjects with stroke in the final study for their Brain Observer Microbleed Scale (Cordonnier et al. 2008).

The final cohort in our project was 303 unselected consecutive subjects, admitted through an acute stroke unit with a variety of radiological diagnoses as shown in the results (Figure 3.1). Our cohort included all common stroke subtypes and some stroke mimics as well. This sample was therefore representative of an acute stroke unit population.

4.1.2: MRI Sequence

The optimum sequence to study microbleeds has not been systematically investigated. Tatsumi argued in a single case report, that the high TE value of GRE MRI sequences may increase the number and size of microbleeds (Tatsumi et al. 2008). A wide range of different TE values (TE = 15 msec, 20 msec, 26 msec, 30 msec and 50 msec) have been used (Cordonnier et al. 2008;Jeerakathil et al. 2004;Lee et al. 2005;Lee et al. 2007;S S et al. 2008;Vernooij et al. 2008a;Vernooij et al. 2008b) (table 1). We, therefore, tested the rating scale on two GRE MRI sequences with different TE values i.e., TE = 40 msec (primary analysis, 273 subjects) and TE = 26 msec (sub analysis, 30 subjects) in this project; to determine whether alteration in the sequence parameters influences intra and inter-rater agreement.

4.1.3: Definition of Brain Microbleeds

The definition of microbleeds has not been consistent for either the size or the morphological appearance in previous studies, as discussed earlier (table 1). We clearly defined microbleeds on the basis of both the appearance and the size (see

methods), which included the whole range of sizes of microbleeds documented in the published literature. Because of difficulties in identifying microbleeds we also classified them into two groups i.e., definite and possible, as discussed in the methods. This gave a high intra and inter-rater agreement, perhaps because it clarified and formalized in the rater's mind the decision making process regarding whether a given lesion is a definite microbleed or a mimic.

4.1.4: Variable Experience of Raters

In only a few previously published studies, the experience of raters of microbleeds on MRI has been mentioned. In these studies the raters were quite experienced (2.5 years experience) in identifying microbleeds (Vernooij et al. 2008b). In our project, the raters had variable range of experience (Rater 1: 1 year, Rater 2: 1 month) to identify microbleeds. These findings suggest that our scale may be applicable to observers with a range of experience.

4.2: Microbleeds in any Location of the Brain at TE=40 msec

4.2.1: Prevalence and Number

In our cohort of subjects, 31.13% to 38.46% had microbleeds (Table 4). As expected this prevalence is higher than that in healthy populations as described in previous studies (Cordonnier et al. 2007;Jeerakathil et al. 2004;Roob et al. 1999;S S et al. 2008;Vernooij et al. 2008b). The higher prevalence in our sample is because the majority of subjects were older (> 60 years) and had risk factors for the development of microbleeds including white matter disease, ischemic infarcts and ICH. It was also noticed that the rater 2 documented more microbleeds in both the ratings than rater 1. We presume that as rater 1 had more experience in rating microbleeds, she was more stringent in documenting them as compared to rater 2.

4.2.2: Agreement for the Presence of Brain Microbleeds

There was good to very good inter and intra-rater agreement for the presence of definite and total (total = definite + possible) microbleeds in any location of the brain. The agreement was fair to good for possible microbleeds (table 5). It is presumed that the agreement for possible microbleeds is less because they do not have a standardized definition and are difficult to differentiate from the mimics e.g., sulcal vessels, basal ganglia calcifications, iron deposits and air-bone interfaces.

It is difficult to compare our scale results with other studies, since intra and inter-rater agreement will depend not only on the properties of the rating scale but also the experience of the raters, patient population and MRI sequences. Nevertheless our results do compare favorably with previous studies.

Our intra-rater agreement for the presence of definite microbleeds was the same as calculated by Vernooij (Vernooij et al. 2008b), but was lower than that found by Sveinbjornsdottir (S S et al. 2008). Sveinbjornsdottir analyzed 19 patients with in the same week (S S et al. 2008) and Vernooij did not mention the gap between the two ratings (Vernooij et al. 2008b) for the assessment of intra-rater agreement. In our project, rater 2 analyzed 303 subjects with a gap of 4 weeks between the 1st and the 2nd rating to calculate the intra-rater agreement. We argue that this may be more representative of serial evaluations in longitudinal studies. The intra-rater agreement for the presence of total microbleeds was less than that for definite ones, because total microbleeds include both definite and possible microbleeds, and possible microbleeds had lower agreement.

Our inter-rater agreement for the presence of definite microbleeds was higher than that found by Jeerakathil (Jeerakathil et al. 2004), Kwa (Kwa et al. 1998) and Cordonnier (Cordonnier et al. 2008) (Table 10). This may be because we used a precise definition of microbleeds and a higher TE value for GRE MRI. The inter-rater agreement for the presence of definite microbleeds was less than that calculated by Fan (Fan et al. 2004), Sveinbjornsdottir (S S et al. 2008), Lee (Lee et al. 2005;Lee et al. 2007), Vernooij (Vernooij et al. 2008a;Vernooij et al. 2008b) (Table 10). The

rating of microbleeds is a rater dependent process, and higher experience of the neurologists and neuroradiologists who rated the MRIs in the above mentioned studies may have had an effect. The inter-rater agreement for the presence of total microbleeds was again less than that for definite microbleeds, for the same reasons as discussed for intra-rater agreement.

It was observed that both the raters had disagreement in those cases where there was only one microbleed. In these cases of disagreement, rater 2 identified more cases with only one microbleed than rater 1. Moreover, rater 1's readings for these cases matched with the arbitrator's readings more often than rater 2. We, therefore, recommend caution in rating microbleeds if there is only one microbleed present.

4.2.3: Agreement for the Number of Brain Microbleeds

The intra and inter-rater agreement for the number of definite microbleeds was moderate to good, and fair to moderate for possible and total microbleeds (appendix1). The agreement for the number was lower than for the presence of microbleeds, probably because, number is a continuous variable and presence or absence is a dichotomous variable. There is only one study by Vernooij and co-workers where inter-rater agreement for the number of microbleeds was calculated. Our agreement was lower in comparison to the agreement described by them (Vernooij et al. 2008a). The possible reason may be that the raters in Vernooij's study had 2.5 years experience in rating microbleeds where as our raters had less experience.

Table 10: Comparison of Intra and Inter-rater Agreement with Previous Studies

	(Kwa et al. 1998)	(Fan et al. 2004)	(Jeerakathil et al. 2004)	(Lee et al. 2005)	(Lee et al. 2007)	(S S et al. 2008)	(Vernooij et al. 2008a)	(Vernooij et al. 2008b)	(Cordonnier et al. 2008)	Current Project
Inter-rater Agreement Kappa (κ) Value	0.6	0.75	0.33 to 0.57	0.87	0.86	0.71 to 0.73	2DT2*WGRE 0.80 3DT2*WGRE 0.85	0.85	0.68	0.69
Intra-rater Agreement Kappa (κ) Value	-	-	-	-	-	1.0	-	0.87	-	0.87

4.3: Brain Microbleeds in Lobar, Deep and Infratentorial Distribution at TE=40 msec

Although a few other studies have described the distribution of microbleeds in the lobar, deep and infratentorial regions (Jeerakathil et al. 2004;Roob et al. 1999), these were conducted in healthy populations or patients with specific types of cerebrovascular disease, and were not representative of an unselected acute stroke population. In our project, the majority of patients had microbleeds (definite, possible and total) in a lobar distribution followed by deep and infratentorial regions (table 6). The number of microbleeds was also the highest in lobar region (Figure 3.3).

4.3.1: Agreement for the Presence of Brain Microbleeds

The agreement for the presence of definite (table 7) and total (definite + possible) (appendix 1) microbleeds in lobar, deep and infratentorial distribution was good to very good, and, as expected lower for the possible microbleeds (appendix 1). The lower agreement for the presence of possible microbleeds was because of the difficulties in identifying them as discussed earlier.

The inter-rater agreement for the presence of total microbleeds (appendix 1) in the lobar, deep and infratentorial regions was good. It was higher than the inter-rater agreement for the combined certain and uncertain microbleeds in the same regions, as found by Cordonnier (Cordonnier et al. 2008). For the presence of definite lobar, deep and infratentorial microbleeds, our inter-rater kappa values had the same range as documented by Cordonnier for the same regions (Cordonnier et al. 2008).

4.3.2: Agreement for the Number of Brain Microbleeds

To investigate how microbleeds may relate to clinical or imaging measures, reliable quantification of the number of microbleeds in different regions is required. The intra and inter-rater agreement for the number of definite and total microbleeds ranged from moderate to very good. It is assumed that the agreement for the number was lower than for the presence of microbleeds because of the nature of two variables, as discussed above. Moreover, two different raters may identify different numbers of microbleeds in the same subject. Possible microbleeds had very low agreement for their number in the lobar, deep and infratentorial regions because of the reasons already mentioned.

4.4: Definite Brain Microbleeds in Individual Anatomical Regions at TE=40msec

4.4.1: Number and Prevalence

The highest number of definite microbleeds was documented in the temporal lobes in our study (figure 17). Few other studies have investigated the distribution of microbleeds in anatomical regions, and these were either in healthy populations (S S et al. 2008), or in specific disease groups, e.g. CAA (Lee et al. 2007). Disagreement between Rater 1 and 2 for the highest prevalence of definite microbleeds out of frontal, temporal, parietal and occipital lobes may be attributed to the air-bone interfaces around frontal lobes and the experience of the raters to differentiate the mimics from definite microbleeds.

4.4.2: Intra and Inter-rater Agreement

The intra and inter-rater agreement for the presence of definite microbleeds was good to very good for the frontal, temporal and parietal lobes, thalamus and brainstem. The intra and inter-rater agreement was moderate to good for the occipital lobes,

lentiform and cerebellum. The artefacts in the posterior fossa due to air-bone interfaces may be misinterpreted as microbleeds. This may be the reason for a moderate inter-rater agreement for the occipital lobes and the cerebellum. In the lentiform nucleus, the presence of calcification and iron deposits may also lead to uncertainty about microbleed rating which could explain the moderate inter-rater agreement. We recommend that CT scans should be consulted for the doubtful cases of microbleeds in basal ganglia to rule out calcifications. Intra and inter-rater agreement were not calculated for insula, centrum semi ovale, external capsule, corpus callosum, caudate and internal capsule, because there were only a few cases with microbleeds in each of these locations.

4.5: Sub Analysis: Agreement for Brain Microbleeds at TE = 26msec

In the sub analysis performed on 30 subjects with GRE MRI at TE = 26 msec the intra and inter-rater agreement for definite, possible and total microbleeds in any location of the brain was good to very good. Although this was a relatively small sample, the results suggest that our rating scale can be applied to different GRE MRI sequences.

4.6: Implications of the rating scale

Microbleeds are an important emerging biomarker for pathologies affecting small vessels, and may be a prognostic risk factor for CVD. In the past few years the clinical implications and significance of microbleeds have been extensively investigated, but some of the key questions remain unanswered. These include the effect of microbleeds on intracerebral bleeding risk and the choice of antithrombotic or thrombolytic treatment; the role of microbleeds in diagnosing CAA versus hypertensive angiopathy in life, and the relationship of microbleeds to cognitive impairment in VD and AD. Answering these questions presents a challenge to researchers and requires standardization of imaging methods and analysis and high quality study design. A key component of any successful study will be a way to

reliably rate the presence, number and anatomical distribution of microbleeds on GRE MRI.

In this project, we used an anatomical rating scale, with a clear definition of microbleeds, on different GRE MRI sequences, in a representative population of stroke patients, and the rating scale was applied by raters with variable experience. We found a good to very good intra and inter-rater agreement for the definite microbleeds in any location of the brain and in individual anatomical regions of the brain. We propose that our scale can, therefore, be used in microbleed studies with different MRI sequences and rater experience. Using this scale, the presence and the number of microbleeds in a single observation as well as in repeated observations can be reliably documented, making it suitable for longitudinal studies. In this way, the question of antithrombotic or thrombolytic treatment in patients with microbleeds can be explored. Moreover, utilizing the ability of this rating scale to categorize microbleeds in anatomical regions may also prove very helpful to study their distribution in CAA and their relationship with cognitive impairment in VD and AD. The next step should be testing our rating scale in different patient cohorts and in different centers to establish its external validity.

5. Conclusion

In summary, this brain microbleed rating scale has good intra and inter-rater agreement for the definite microbleeds in any location of the brain and in individual anatomical regions. We recommend that this scale should be further investigated in longitudinal and cross-sectional studies in different clinical population and centers.

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